TRANSMISSION AND HOST RANGE STUDIES WITH EMPHASIS ON WEED HOSTS OF SUNFLOWER NECROSIS VIRUS DISEASE

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TRANSMISSION AND HOST RANGE STUDIES WITH EMPHASIS ON WEED HOSTS OF SUNFLOWER NECROSIS VIRUS DISEASE

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in partial fulfilment of the requirements
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CERTIFICATE

This is to certify that the thesis entitled **"TRANSMISSION AND HOST RANGE STUDIES WITH EMPHASIS ON WEED HOSTS OF SUNFLOWER NECROSIS VIRUS DISEASE"** submitted by **Ms. ANJULA, N.** in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) IN PLANT PATHOLOGY**, to the University of Agricultural Sciences, Bangalore is a record of research work done by her, during the period of this study in this University, under my guidance and supervision and the thesis has not previously been formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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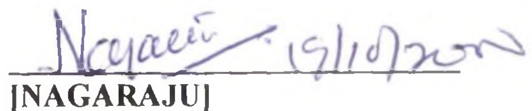

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INTRODUCTION

I INTRODUCTION

Oil seed crops play an important role in the national economy of the country and ranks second after food grains. Oils, not only form an essential part of human diet but also serve as important raw material for agro-based industries.

Sunflower (*Helianthus annuus* Linn.), is one of the important oil seed crops of the world and ranks third, next only to soybean and groundnut. It has been grown for ornamental purpose, oil production, poultry feed and in medicine as diuretic and for treating certain disorders of the respiratory tract (Putt, 1978 ; Lofgren, 1978; Dorrell, 1978). Fried sunflower with pepper is a delicacy. The seed flour being highly nutritive, is also used in bakery. The seeds provides a purple dye for basketary and textiles as well as for body paint in the SouthWest (Whiting, 1939). The stems are used to construct ventilated hood for the piki-stone. Jenness (1958) and Harvard (1985) mentioned the use of sunflower oil to anoint the hair and skin and sunflower used as bird seed in Asia, Australia, Africa, Europe, North America and South America (Putt and Sackston, 1957). The cultivated sunflower (*Helianthus annuus* var. Macrocarpus (DC) Ckll.) is among the four most important annual crops in the world grown for edible oil (Putt, 1978). Its domestication as an oil seed crop started in sixteenth century in Europe. Its cultivation spread to other parts of the world during 1960's after the development of varieties in former USSR possessing with high seedand oil yields. In the recent past the cultivation of this crop has been considerably extended to Australia, USA and India (Carter, 1978; Kolte, 1985).

In India, the importance of sunflower as an oil seed crop was realised very recently, although it is being grown since long time as an ornamental crop. Due to its desirable attributes viz., high potentiality of yielding quality edible oil, ability to adjust as an inter crop, drought tolerance, short duration habit, better performance under varied soil and climatic assortments, this crop is gaining importance year after year.

The oil content in the sunflower seed is about 40-45 per cent with high amount of poly un-saturated fatty acid (44-76 % linoleic acid and 14-43 % oleic acid) (Chatrapathi, 1973). It is considered to be a high quality edible oil for health point of view, because of low cholesterol properties and anti-cholesterol properties of oil. Being a semi drying oil, it is used in paint and varnish industries. The oil cake is employed in the manufacture of live stock feeds and fertilizers. In recent years, the entire plant is widely used for making silage and as fuel.

The area under sunflower at global level is about 17.64 m. ha with the production of 21.64 m t with productivity of 1227 kg/ha. In India, it is grown over an area of about 2.00 m. ha annually producing 12.32 m t. with productivity of about 656 kg/ha (Anon, 1998b).

Karnataka is one of the leading sunflower growing states in India with an area of 0.88 m. ha and production of 0.27 m. t and productivity of 420 kg/ha (Anon, 1998b).

Sunflower is reported to be susceptible to several diseases caused by various agents. The most important ones being Alternaria leaf spot

(*Alternaria helianthi* Hansf.), rust (*Puccinia helianthi* Schori.) and downy mildew [(*Plasrmopara halstedii* Fari.) Berl. and de toni]. Many virus diseases have also been reported to reduce the seed yield from several countries (Kolte, 1985). Several virus and virus like diseases viz., sunflower mosaic, rugose mosaic, yellow ring mosaic, yellow mosaic, yellow spot, chlorotic leaf mosaic, greening cucumber mosaic, curl mosaic and mycoplasma like organism (MLO) (Strains of tomato big bud, stolber, aster yellows and phyllody) have been reported to infect sunflower (Nagaraju, 1995).

Very recently, a new virus disease has been reported to occur on sunflower showing necrosis on leaves, stem, calyx and heads, showing mosaic, chlorotic spots/ring spots on leaves, malformation of leaves and heads, severe stunting of plants, causing severe yield losses around Bangalore (Anon, 1997; Singh *et al.*, 1997) because of its fast spreading nature and severity. The new virus is considered as one of the threatening diseases of this crop (Nagaraju *et al.*, 1997).

The preliminary studies conducted, revealed the disease is of viral in nature (Jain *et al.*, 2000). Further, the transmission of causal agent was successfully established through mechanical sap inoculation by Anil Kumar (1999) and also through vector thrips by Shivasharanayya (2000).

However, the detailed investigations regarding the host range of the causal agent in general and the weed hosts of the virus in particular have not been attempted and are totally lacking.

Keeping the above points in view on this important virus disease, the studies were under taken with the following objectives:

1) To make Survey for the incidence of sunflower necrosis virus disease in and around Bangalore.

2) Transmission studies of the disease through mechanical sap, insect vector and seeds and

3) Host range of the disease with emphasis on weed hosts prevailing around the field.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Sunflower is reported to be susceptible to several diseases (Kolte, 1985). Among them, the virus and virus-like organisms which have been reported to occur on this crop are,

* Sunflower mosaic virus (SMV) (Orellana and Quacquarelli, 1968)
* Aphid transmitted yellowing virus disease of sunflower (AYVD) (Russel *et al.*,1975)
* Sunflower ring spot virus (SRSV) (Behncken, 1974)
* Yellow blotch (YBD) and leaf crinkle disease (LCD) (Theuri and Bock, 1986; Theuri *et al.*, 1987)
* Tobacco streak virus on sunflower (TSV) (Dijkstra, 1983; Kaiser *et al.*, 1982)
* Tobacco leaf curl virus (Tob LCV) (Chatterjee and Pandey, 1974, 1977)
* Sunflower mosaic virus caused by a strain of cucumber mosaic virus (SMV-C) (Arnott and Smith, 1967; Orellana and Quacquarelli, 1968; Gupta *et al.*, 1977; Gupta, 1981; Gupta and Roy, 1981; Venugopal Rao *et al.*, 1987; Jitendramohan, 1992; Nagaraju *et al.*, 1997)
* Aster yellow (MLO) (Severin and Frietag, 1945; Sackston, 1958) and
* Phyllody (MLO) (Nour, 1962; Bidari *et al.*, 1987).

Recently, a new virus disease on sunflower showing necrotic symptoms causing severe yield losses was reported to occur around Bangalore (Anon, 1997; Singh *et al.*, 1997). However, the occurrence of

the causative agent and its detailed characterization have not been made or reported elsewhere in the world. In this chapter a brief review of virus and virus like diseases of sunflower occurring in India are presented. Tospoviruses occurring on other crops like watermelon, groundnut, tomato and impatiens are also presented here.

Sunflower mosaic virus (SMV)

Weber (1932) for the first time reported a mosaic disease of sunflower from Florida. In India a mosaic disease of sunflower was reported for the first time but the viral nature was not confirmed (Uppal, 1933).

Battu and Phatak (1965) observed a mosaic disease on sunflower in the premises of Indian Agricultural Research Institute, New Delhi, showing mosaic symptoms accompanied by ring spots or chlorotic spots which had a tendency to coalesce. The disease was sap transmissible and produced typical viral symptoms ten days after inoculation. They failed to transmit the virus to any other host plant except sunflower.

Orellana and Quacquarelli (1968) reported a mosaic disease on sunflower caused by a strain of cucumber mosaic virus from Beltzville, Maryland, USA. The disease was characterized by mosaic chlorotic rings on young leaves of two months old plants. Further, diseased plants produced malformed heads and shrivelled seeds. They transmitted the virus by sap inoculation using 0.1 M phosphate buffer pH 7.0 with carborundum

as abrasive. Further, the virus produced leaf mottling on *Aster chinensis* and *Cassia occidentalis* L. *Chenopodium amaranticolor* Coste and Regn., produced necrotic local lesions. *Cucumis sativus* L. cv. Zucchins squash produced chlorotic local lesions and mosaic *Datura stramonium* L. expressed chlorotic local lesions, *Lycopersicon esculentum* Mill. cv. Rutgers produced leaf crinkling and veinal necrosis *Nicotiana tabacum* L. cvs. Samsun 402, Kentuckz 35 and *Nicotiana rustica* L., produced vein yellowing. *Vigna sinensis* L. cv. , Black Eye produced raddish local lesions followed by mosaic symptoms.

Gupta (1981) reported sunflower mosaic virus from India which produced small circular, chlorotic spots on leaves, chlorotic spots on leaves which coalesced to form typical mosaic pattern cupping and malformation of leaves with poorly developed root system, reduction in number of seeds per head, seed viability and pollen sterility were also associated with the disease. The disease was shown to be sap transmissible in phosphate buffer with Na_2So_3. The virus was also graft and seed transmitted. Further, *Bemisia tabaci* generally transmitted the virus with one hour acquisition and six hour inoculation feeding period.

The virus was restricted to *Helianthus annuus* and *Amaranthus* spp., producing systemic symptoms and *Chenopodium amaranticolor* reacted with chlorotic local lesions.

Venugopal Rao *et al.* (1987) from Hyderabad, India, reported a strain of cucumber mosaic virus infection on sunflower. The virus was sap transmissible with ease and produced mosaic mottling accompanied by

concentric chlorotic rings on young leaves of inoculated plants. The virus was not seed transmitted but was transmitted by three aphid species namely, *Aphis gossypii* Glover, *Aphis craccivora* Koch., and *Rhopalosiphum maidis* in a non persistant manner with 70, 50 and 30 per cent transmission respectively. Further, virus infected *Zinnia elegans* Benth., *Carthamus tinctorius* Linn., *Nicotiana tabacum* cvs., Harrisons special, Xanthi NC and Havana 307, *Nicotiana glutinosa, Capsicum annuum* Linn., *Solanum melongena* Linn., *Lycopersicon esculantum* Mill., *Cucumis sativus* Linn., *Cucumis melo* Linn., *Dolichos lablab* Linn., *Chenopodium amaranticolor* Cost and Reyn., *Gomphrena globosa, Mamordica charantia, Chenopodium album* Linn., and *Luffa accutangufolia* Roxb.

Jitendramohan (1992) from Meerut, reported another strain of cucumber mosaic virus infecting sunflower which produced chlorotic rings and mosaic mottling. The virus was transmitted by sap and also aphid vector namely, *Myzus persicae* Sultz., *Aphis gossypii* Glover, *Aphis malvae, Aphis nerii* and *Aphis fabae* in a non-persistant manner. The virus was found to be transmitted to 135 species of plants belonging to family solanaceae, 6 species of cucurbitaceae and 2 species of cruciferae. On *Chenopodium amaranticolor,* it produced local lesions as white dots, on *Nicotiana tabacum* cv. White Burley reacted with local necrotic lesions. They also described *Amaranthus viridis* Linn., *Nicotiana plumegi-folia* Linn., *Stellaria media* and *Sonchus oleraceous* as natural weed hosts of the virus.

Nagaraju (1995), for the first time reported a sap transmissible virus disease on sunflower from Karnataka producing chlorotic ring spot and mosaic symptoms and designated it as sunflower mosaic virus (SMV). The virus was transmitted through mechanical sap (upto 40 per cent) and also by aphid vector viz., *Aphis gossypii* (20-24 per cent), *Aphis craccivora* (14-16 per cent) and *Myzus persicae* (20-25 per cent). The virus had a dilution end point of 1:500-1:1000, thermal inactivation point of 55-65^{0}C and longevity *in vitro* for 66-72 hr at room temperature (21-28^{0}C). Further the virus had a very narrow host range infecting only one weed host *Galinsoga parviflora* in addition to the sunflower cultivars.

Yellow Ring Mosaic Virus

Gupta and Gupta (1977) reported a yellow ring mosaic disease from India which produced severe mosaic symptoms, stunting of plants and malformation of young leaves. The virus was transmissible by sap and by leaf and leaf grafting. Standard sap extract prepared with Na_2SO_3 (0.1 per cent) and carborundum 600 mesh as abrasive gave best results. They further said that the virus has a limited host range producing chlorotic local lesions on *Chenopodium amaranticolor* only.

Tobacco Leaf Curl Virus (TLCV)

Chatterjee and Pandey (1974) reported tobacco leaf curl virus (TLCV) on sunflower from India. Further, they tested one hundred and eight genotypes of sunflower for their reaction to TLCV both under field conditions and Insect proof glass house at Indian Agricultural Research

Institute, Regional Station, Pune, using whitefly, *Bemisia tabaci* (Genn.). Out of 108 genotypes, six namely, Sunrise selection EC 50229, EC 50277, EC 54641, EC 68414 and EC 97923 were reported to be susceptible to infection in glass house with transmission ranging from 8.32 to 25.0 per cent. Whereas, under field conditions, Sunrise selection EC 50277 and EC 54641 were only susceptible expressing 16.6 to 33.3 per cent infection. Remaining 102 genotypes were not infected even under glass house conditions.

Phytoplasma Disease

Phyllody

The first report of occurrence of phyllody on sunflower was made by Nour in 1962 from Sudan. The infected plants remained stunted with the leaves showing many zonal necrotic areas. Such plants produced abnormal heads, with floral parts transformed into green leaf like structures.

Signoret *et al.* (1976) reported the presence of pleomorphic bodies of 200-800 nm diameter in the phloem tissues of infected plants.

Klein (1970), from Israel reported that phyllody of sunflower under Israel conditions also caused witches broom, yellowing, sterility and further, the disease was reported to be transmitted by a leaf hopper, *Neoalitirus fenestratus* Herrich-Schaffer.

Bidari *et al.* (1987) reported a phyllody disease on sunflower from Karnataka, India and described the symptoms as small prominent cholrotic

leaves which later turned necrotic. Infected plants remained green for longer time. The capitulum, instead of producing normal ray and disk florets, showed wedge shaped sectors with hypertrophied flowers. The ovary, calyx, corolla and another tuber showed green coloured short rudimentary leaves. In early infections, plants remained stunted with small abnormal capitulum without ray florets. The infected plants later showed light green ray florets and produced hypertrophied disk florets.

Sunflower Necrosis Virus Disease

A new virus disease on sunflower with necrotic symptoms causing severe yield losses was reported to occur around Bangalore (Anon, 1997; Singh *et al.*, 1997). The disease was noticed for the first time in the country during 1997 at Bagepally of Kolar district (Karnataka) in seed production plots. Earlier to sunflower necrosis disease, there was an epidemic of watermelon bud necrosis in the same area. Subsequently, the disease has been reported through out the state of Karnataka, Coimbatore, North and South Arcot districts of Tamil Nadu ; Hyderabad, Rangareddy, Kurnool districts of A.P. Recently, the disease was observed in the districts of Jalna, Aurangabad, Latur and Akola of Maharashtra and because of its fast spreading nature, it was considered as one of the threatening diseases on this crop (Nagaraju *et al.*, 1998).

Symptomatology

The symptoms of the disease appeared as necrosis of leaf lamina which extended through the petiole to the main stem and to flower buds. The buds from the infected plant were twisted partially and became sterile.

The flower buds failed to expand or enlarge after flowering. The internodal length was reduced resulting in dwarfening of plants. However, some plants showed recovery and produced normal flowers which were of smaller size, systemic symptoms like mosaic mottling and yellowing accompanied by small earhead and necrotic calyx, corolla and other floral parts were also observed (Nagaraju *et al.*, 1998). Plants at all stages of growth were found to be susceptible.

Symptomatological observations under field conditions reveal that disease symptoms appeared as sudden necrosis of part of leaf lamina, necrosis extending as black streak through the petiole and to the terminal shoot. The infected plant and flower buds were partially twisted which later became sterile. Systemic symptoms after initial necrosis developed as various types of mosaic mottling, puckering, twisting of leaves narrowing and yellowing. Such systemically infected plants remained stunted. Some plants were found to recover after initial symptom of necrosis which are shorter compared to healthy ones. Such plants were initially stunted due to reduced internodal length and later become normal producing flowers and seeds (Anil Kumar, 1999; Shivasharanayya, 2000).

Halakeri (1999), reported that symptoms under field condition appeared as mosaic, chlorotic rings/streaks, marginal necrosis and stunting of plants. Severe stunting, necrosis and distortion of young top leaves were the symptoms recorded in early infected plants, which produced small malformed heads whereas late infected ones produced partially filled heads with shrivelled seeds. The disease incidence was more in *rabi* season compared to *kharif*.

Survey

The incidence of the disease ranged from 5.35 to 21 per cent, incidence highest being in May-June sown crop but declined after the onset of rains during kharif (Anon., 1997). The disease incidence was reported from several parts of Karnataka i.e. Dharwad, Raichur, Chitradurga, Haveri, Ranibennur, Naragund, Gadag, Tumkur and Kolar (Anon, 1998a).

In Raichur district 7 to 36 per cent incidence of the necrosis disease was recorded around Raichur, Manvi and Devdurg during kharif 1998 (Anon, 1999).

Fifteen to twenty per cent incidence of necrosis disease has also been recorded around north Arcot in Coimbatore district of Tamil Nadu state during survey undertaken in August to September 1998 (Anon, 1999).

Similarly the necrosis disease has been recorded to a tune of two per cent around Latur and Osmanabad districts of Maharashtra state during *Kharif* 1998 (Anon., 1999).

Halakeri (1999) reported the presence of disease in almost all parts of the districts surveyed in Dharwad. The incidence ranged three per cent at Kurtakoti to 70 per cent at Radder Timmapur. Incidence and severity was higher in the fields grown with ITC-Zeneca (PAC-36) sunflower hybrid. Maximum disease was observed in Bagalakot district.

Anilkumar (1999) and Shivasharnayya (2000) reported that the sunflower necrosis virus disease was prevalent in all the sunflower fields visited during survey with the maximum per cent incidence (21.1) during the June 1998. It was also observed that the incidence was more in crops sown during summer months followed by *kharif* and *rabi* sown crops.

Characterisation

Tomato spotted wilt virus was identified for the first time in Ukraine by Zakusilo *et al.* (1994) with yellow spot mosaic, necrotic lesions and systemic mosaic symptoms. The particle size reported ranged from 50 to 120 nm (isometric particle). The particles were unstable and were difficult to purify. Further the transmission of this virus was by thrips.

Singh *et al.* (1997) reported the occurrence of sunflower necrosis virus disease in an epidemic form around Bangalore during 1997. The electron microscopic studies of the disease had been made and reported the presence of spherical virus particles measuring 85-90 nm in diameter, showing moderate reaction of this virus with the antiserum of watermelon bud necrosis virus.

Jain *et al.* (2000) studied the serological relationship of sunflower necrosis with the available antisera of the member of "tospovirus"

serogroup at IARI and reported the strong relation of this virus with the antisera of Watermelon Silver Mottle Virus (WSMV) and Peanut Bud Necrosis Virus (PBNV) and concluded that this virus belongs to 'tospovirus' serogroup, occurring in India.

Transmission

Anil Kumar (1999) reported that the disease could be transmitted through sap when potassium phosphate buffer 0.05 M with 0.02 M 2-mercaptoethanol was used but to lower percentage (3.33%). Potassium phosphate buffer (KH_2PO_4-0.01 M and K_2HPO_4-0.03 M) with 0.075% thioglycerol however, increased the percentage of transmission up to 46.6 per cent. Among the anti-inhibitor as evidenced by higher transmission (46.6 %), Carborundum (600 mesh size) and celite (600 mesh size) when incorporated into the extract gave good percentage of transmission ranging from 40 to 46.6 per cent with potassium phosphate buffer.

Seedlings raised from the seeds collected from infected plants of different genotypes did not show any type of disease symptoms indicating the non-seed transmission of the disease. Further, whiteflies (*Bemisia tabaci*), leafhopper (*Amrasca bigutulla bigutulla*), and aphids (*Myzus persicae, Aphis craccivora* and *Aphis gossypii*) failed to transmit the disease under glass house conditions even when high AAP and IAP were given (Anil Kumar, 1999).

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The sap inoculation technique for the virus was standardized using 0.5 M potassium phosphate buffer (pH 7.0) containing 0.1 per cent sodium sulphide as antioxidant. Sunflower necrosis virus was not readily sap transmissible as very low transmission was recorded when the sap inoculations were made to sunflower, cowpea and tomato (Halakeri, 1999).

Among the different insects aphids (*Myzus persicae*), jassids (*Emphovasca kerri*) and white flies (*Bemisia tabaci*) failed to transmit the virus indicating only thrips (*Thrips tabaci*) as vectors responsible for the natural spread of the virus in field. This also gave an clue that trospovirus is the most probable causal agent of the necrosis disease as thrips can transmit only tospovirus. The virus was not transmitted by seed or through dodder also (Halakeri, 1999).

Shivasharanayya (2000) reported that the disease could be transmitted through sap when standard inoculum prepared from potassium phosphate buffer (0.75% thioglycerol) induced the symptoms of chlorotic spots and necrosis of the tip on test plants. The percentage of transmission ranged from 30 to 40. The seedlings raised from the seeds collected from naturally infected plants in the field of different genotypes did not show any type of disease symptoms indicating the non-transmissible nature of the disease through seed.

Shivasharanayya (2000) reported that the vector, *Thrips palmi* karny was found successful in transmitting the disease when a longer acquisition

access period of three days was given under glass house conditions. The per cent transmission ranged from 15.0 to 22.5.

Host range

Out of the 44 plant species tested, the pathogen infected only *Cucumis sativus* cv. Green Long producing chlorotic spots followed by necrosis of the leaves along margin, narrowing of leaves and stunting of plants, under lab condition upon mechanical sap inoculation (Anil Kumar, 1999).

Among the different hosts used as test plant for mechanical transmission studies, the virus infected *Vigna anguiculata* cv. C-152 and *Lycopersicon esculentum* apart from main host *Helianthus annuus* (Halakeri, 1999).

Detailed information regarding the transmission, vector relationship, host range, EM, serology etc., of the disease are lacking. Hence, review relating to the watermelon bud necrosis virus and other related viruses such as groundnut bud necrosis virus, impatience necrotic spot virus (INSV) and tomato spotted wilt, tospovirus (TSWV) have also been reviewed and presented briefly.

Watermelon bud necrosis disease

The disease was first reported in silver mottle diseased watermelon plants from Japan (Iwaki *et al.,* 1984) and was shown to be caused by

tospovirus. Yeh *et al.* (1992) reported a similar virus with different disease symptoms from Taiwan. Under field conditions, watermelon plants exhibited mosaic mottling, crinkling, yellow spotting and narrowing of leaf lamina. Infected plants were severely stunted with short internodes and upright younger branches, tip necrosis and dieback. Fruit set was reduced and the fruit that developed remained small, malformed and showed necrotic spots.

'Bud necrosis' a viral disease of watermelon was observed for the first time during 1991-92 in the crop grown at Indian Institute of Horticultural Research Hesaraghatta Lake and was reported to be caused by a tospovirus (Singh and Krishna Reddy, 1995). The symptoms included necrosis of the buds and petiole, necrotic spots on leaves and necrotic streak on veins. Infected vines were reported to eventually wilt and dry, while fruits from infected plants of few varieties exhibited broken concentric rings with corky texture on the surface (Singh and Krishna Reddy, 1997). The disease was shown to be caused by watermelon strain of tomato spotted wilt virus (TSWV-W) (Krishna Reddy and Singh, 1993).

Chohan (1967) succeeded in transmitting the virus by sap with the addition of 0.02 M 2-mercaptoethanol as an anti-inhibitor in the potassium phosphate buffer (pH-7).

Successful transmission of TSWV could be obtained when young infected leaf tissue was used as a source of inoculum and when the

inoculum was prepared using cold pestle and mortar (Ghanekar *et al.*, 1979).

The tospoviruses were reported to be transmitted by thrips, belonging to the order Thysanoptera, family Thripidae (Sakimura, 1962, 1969; Amin *et al.*, 1981; German and Mayer, 1992; Iwaki *et al.*, 1984; Reddy and Wightman, 1988; Wijkamp *et al.*, 1995; Ullmann, 1996).

Two types of thrips, *Frankliniella schultzei* Tozbom and *Scirotothrips dorsalis* Hord, have been reported to transmit TSWV in India (Ghanekar *et al.*, 1979; Amin *et al.*, 1981). Later, Ranga Rao and Vijaya Lakshmi (1992) showed that *F. schultzei* was a poor vector of peanut bud necrosis virus.

Singh and Krishna Reddy (1995) reported *Thrips flavus* Schrank., as a new vector of tosporivus infecting watermelon plants in India. About 10-15 nymphs with acquisition access period of 3-4 days and about 15-20 days of inoculation feeding successfully transmitted the virus.

TSWV had extremely wide host range exceeding 200 species of plants covering 34 families, almost half of the known hosts belonging to solanaceae (Halliwell and Philley, 1974).

TSWV-W (Watermelon isolate from Taiwan) and TSWV-NV tomato isolate of New York produced similar symptoms on test plants in

Amaranthaceae, Chenopodiaceae and Solanaceae (Yeh *et al.*, 1992). With the exception of watermelon isolates of TSWV from Japan (Iwaki *et al.*, 1984) and Taiwan (Yeh *et al.*, 1992) most tospoviruses induce only local infection on Cucurbitaceous plants (Ie, 1970; Reddy and Weightman, 1988). Singh and Krishna Reddy (1995) showed that the TSWV-W isolate from India systemically infected watermelon, muskmelon and other cucurbits.

Bud necrosis disease of groundnut

This disease caused by tomato spotted wilt virus was first reported as ring spot from Brazil (Costa, 1941). In India occurrence of bud necrosis or bud blight disease in severe forms has been reported from Punjab (Chohan, 1967) and Andhra Pradesh (Reddy *et al.*, 1968).

Under field conditions, the infected plants exhibited distinct chlorotic ring spots or specking with a few necrotic spots or chlorotic leaf spots with green island, necrosis on the petioles, along the stem and on the terminal bud (Ghanekar *et al.*, 1979).

Successful transmission of virus was obtained by using young infected leaves showing chlorotic spots as source of inoculum and when the inoculum was prepared in a chilled extract using cold pestle and mortar (Ghanekar *et al.*, 1979).

PBND is the most important viral disease of groundnut in India until 1977, the vector involved was unknown. Amin *et al.* (1978) reported *Scirtothrips dorsalis* as the vector of the virus that caused PBND. This was confirmed by Ghanekar *et al.* (1979). Later Amin *et al.* (1981) reported the transmission of TSWV by *S. dorsalis* and *F. schultzei. S. dorsalis* was found to be a less efficient vector than *F. schultzei.* Palmer *et al.*, (1990) discovered the occurrence of *Thrips palmi* in groundnut in India and suspected its involvement in the transmission of PBNV. Recently, Vijaya Lakshmi *et al.* (1995) from ICRISAT reported *T. palmi* as major and efficient vector of PBNV.

Only larval stages of thrips could acquire the virus, while both larval and adult thrips were reported to transmit the virus in a persistent manner (Sakimura, 1962).

TSWV was shown to infect over 200 species of plants covering 34 families and almost half of the known hosts of Solanaceae (Halliwell and Philley, 1974).

Ghanekar *et al.* (1979) reported chlorotic and necrotic local lesions due to TSWV on *Beta vulgaris* L. *Chenopodium amaranticolor* Coste and Regn., *Crotalaria juncea* L., *Cucumis sativus* var. National Pickling *Nicotiana rustica* L., *N. tabacum* L., cv. Xanthinc. While, chlorotic or necrotic spots followed by systemic infection was observed on *Dolichos uniflorus* L., *Lycopersicon esculentum* Mill. var. Pusa Ruby, *Phaseolus lunatus* L., *Vigna mungo* L. ar. Vpv-1 and *Zinnia elegans* Benth.

Impatience necrotic spot virus (INSV)

The impatience necrotic spot caused by a tospovirus was reported on impatience from USA (Law and Moyer, 1990). The virus produced necrotic spots with necrotic rings on young leaves while older leaves remained symptom less. Further, the virus was reported to be transmitted by *Frankliniella occidentalis.*

Verheven and Roenhorst (1994) reported INSV on *Chrysanthemum* sp., *Kalanchoe* sp., apart from impatience from Holland. Volvos and Lafortezza (1994) from Italy reported that INSV could also infect Lisanthus (*Eustoma grandiflorum*).

Tomato spotted wilt virus (TSWV)

Tomato tospovirus causing enormous losses limiting the tomato production was reported for the first time from Australia in 1915 (Brittlebank, 1919).

The virus was reported from India for the first time from Nilgiris in Tamil Nadu on tomato var. Marglobe (Todd *et al.,* 1975). The virus was found to produce characteristic necrosis of the young growing bud, bronzing of the leaves with brown necrotic lesions, which spread systemically, followed by wilting of plants. The ripened fruits exhibited spots with circular markings about 1 cm diameter as concentric bands of red and yellow brown rings.

In India tospoviruses have been reported on crops viz., peanut (Ghanekar *et al.*,1979), tomato (Prasad Rao *et al.*, 1984), urdbean and mungbean (Amin *et al.*, 1985) and on cowpea, chilli, brinjal and clusterbean (Prasad Rao *et al.*, 1987).

Sastry (1982) reported the occurrence of tomato tospovirus on tomato from Karnataka, with symptoms of tip necrosis, necrotic spots on leaves, petioles and stems of the infected plants and wilting. Infected plants produced fruits with chlorotic concentric rings.

Symptoms of bronzing, severe necrosis of leaves and growing buds leading to die back and death of plants was reported from Andhra Pradesh. The incidence of the disease ranged from 1.58 to 18.51 per cent during rabi 1978, whereas it was 100 per cent in kharif 1979 (Prasad Rao *et al.*, 1980).

Chohan (1967) reported successful transmission of the virus by sap inoculation using potassium phosphate buffer (pH 7.0) with the addition of 0.02M 2-mercaptoethanol as an anti-inhibitor. Use of sodium sulphite (0.05 per cent) in the buffer was reported to give inconsistent transmission (Ghanekar and Nene, 1976). Successful transmission of TSWV was obtained by using young infected leaf tissue, inoculum prepared in cold using cold pestle and mortar (Ghaneakr *et al.*, 1979).

Prasad Rao *et al.* (1980) found that the use of cold 0.5M potassium phosphate buffer pH 7.0 containing 0.02 M 2-mercaptoethanol was suitable for successful transmission of the virus.

Cho *et al.* (1986) listed 237 host plants susceptible to TSWV from different parts of the world.

TSWV has been reported to infected more than 500 species of plants in more than 50 families including important ornamental, fruit and vegetable crops. More than 100 species of plants in Solanaceae and Compositae alone were found susceptible (Peters *et al.*, 1996; Stobbs *et al.*, 1992). Many of these plants are hosts for the virus and serve as reservoirs of infection that contribute to epidemics in crop plants (Cho *et al.*, 1986).

Tospovirus are known to be vectored by thrips. Pitman (1927) was the first to establish that onion thrips (*Thrips tabaci*) transmitted TSWV in tomato. Seven other species of thrips viz., *Frankliniella fusca, F. occidentalis, F. schultzei, Scirtothrips dorsalis, Thrips palmi, T. sefosus* and *F. temuicornis* have been established as vectors of tospovirus (Kormelink, 1994). Recent work on groundnut in India (Palmer *et al.*, 1990) indicated that *T. palmi* could be a vector of PBNV. Vijay Lakshmi (1994) proved that *T. palmi* was the main vector of PBNV both under field and laboratory conditions.

Varying acquisition inoculation thresholds and latent periods of virus transmission by thrips have been reported (Sakimura, 1962). Recent evidence suggests that viral replication may be an important determinant of thrips infectivity (Ullmann*et al.*, 1992). Several species of thrips currently known to vector TSWV are listed below.

Table 1 : Species of thrips currently known to be the vector of TSWV

Host Plant	Vector	Reference
Tomato	*Thrips tabaci*	Pittman (1927)
Truck crops and Ornamentals	*Franklineila occidentalis* *T. tabaci*	Garnder *et al.* (1935)
Tomato and lettuce	*F. occidentalis* *F. fusca*	Sakimura (1961)
Tobacco	*F. fusca*	Sakimura (1963)
Aster. Emilia and Tomato	*F. fusca*	Paliwal (1974)
Tobacco	*T. tabaci*	Zawirska (1981)
Greenhouse crops	*F. occidentalis*	Stenier and Elliott (1983)
Dahlia, *Lycopersicon, Sinnigi and Tagetis* spp.	*F. occidentalis*	Allen and Broadbent (1986)
Greenhouse crops	*F. occidentalis*	Broadbent *et al.* (1987)
Water melon	*Thrips palmi*	Iwaki *et al.* (1988)
Ageratum, Begonia Calendulla, Cyclamen, Cineraria, Dalhia, Glosinia, *Guinea impatiens,* Marigold, Primerose Tomato	*F. occidentalis*	Matteoni *et al.* (1988)
Groundnut	*F. occidentalis*	Stewart *et al.* (1989)
Cinearia, Calceoloria, Salvia, Capsicum and *Galinosoga parviflora*	*F. occidentalis*	Gofflot and Verhoyen (1990)

Table 1 : (Contd....)

Host Plant	Vector	Reference
Anemone coronaria Ramunculus asiaticus, Eustoma genadiflorum, Pepper and Tomato	*F. occidentalis*	Lisa *et al.* (1990)
Pepper, Tomato, Eggplant Broadbean, Lettuce, Basil, Chrysanthemum, Newguinea, Anemone and Gloxiana	*F. occidentalis*	Marchoux *et al.*, (1991)
Groundnut	*F.schultzei* *F. occidentalis*	Mulder *et al.* (1991)
Grass-pea	*F. occidentalis*	Rogijo and Habers (1992)
Watermelon	*T. Palmi*	Shyivji-dong Yeh *et al.* (1992)
Weeds and native plants of Canada	*F. occidentalis*	Stobbs *et al.* (1992)
Tomato and Capsicum	*F. occidentalis*	Verhoeven and Roenjohrst (1992)
Capsicum annuum and *Sonchus asper*	*F. fusca*	Hobbs *et al.* (1993)
Groundnut	*T. palmi*	Vijaya Lakshmi *et al.* (1995); Weightman *et al.* (1995)
Tagetis spp. Greenhouse crops	*F. occidentalis*	Broadbent *et al.* (1987)

Ananthakrishnan (1971) reported the occurrence of five species of thrips on sunflower viz., *Thrips flavus, Scirtothrips dorsalis, Frankliniella schultzei, Thrips tabaci* and *Caliothrips indicus.*

Table 2 : Tospovirus occurring in India

Sl. No.	Disease	Crop	Virus	Transmission			Reference
				Mechanical	Seed	Vector	
1	Top Necrosis	Chilli	Tomato Spotted Wilt Virus (TSWV)	+	NR	NR	Bidari, 1984
2	Tip Necrosis	Cluster bean	-do -	+	NR	NR	Krishna Reddy and Varma, 1990
3	Tip Necrosis and Leaf curl	Cowpea	-do-	+	NR	NR	Prasad Rao *et al.*, 1987
4	Chlorotic spot	Egg plant	-do-	+	NR	NR	Prasad Rao *et al.*, 1987
5	Leaf curl	Mungbean	-do-	+	NR	*Frankliniella schultzei*	Amin *et al.*, 1985.
6	Tip Necrosis	Pea	-do-	+	NR	NR	Prasad Rao *et al.*, 1984
7	Bud Necrosis	Peanut	Peanut Bud Necrosis Virus (PBNV)	+	-	*Thrips palmi, Scirtothrips dorsalis*	Ghanekar *et al.*, 1979; Vijaya Lakshmi *et al.*, 1995
8	Spotted wilt	Peanut	Tomato Spotted Wilt Virus (TSWV)	+	NR	*Frankliniella Schultzei*	Amin *et al.*, 1981; Ghanekar *et al.*, 1979
9	Yellow spot	Peanut	Peanut Yellow Spot Virus (PYSV)	+	NR	*Scirtothrips dorsalis*	Reddy *et al.*, 1991; Sathyanarayana *et al.*, 1996.
10	Bud blight	Soybean	Peanut Bud Necrosis Virus (PBNV)	+	NR	NR	Thakur *et al.*, 1996.
11	Spotted Wilt	Tomato	Tomato Spotted Wilt Virus (TSWV)	+	-	*Scirtothrips dorsalis, Thrips palmi*	Prasad Rao *et al.*, 1980; Todd *et al.*, 1975.
12	Leaf Curl	Urdbean	Peanut Bud Necrosis Virus (PBNV)	+	NR	*Frankliniella Schultzei*	Amin *et al.*, 1985.
13	Bud Necrosis	Watermelon	Watermelon Bud Necrosis Virus	+	-	*Thrips palmi*	Singh and Krishna Reddy 1997.
14	Necrosis Virus	Sunflower	Sunflower Necrosis Virus (SNV)	+	-	NR	Nagaraju *et al.*, 1998.
15	Potato Stem Necrosis	Potato	Potato Stem Necrosis Virus	+	NR	NR	Khurana *et al.*, 1998.

NR = Not recorded

Table 3 : Thrips species transmitting different tospovirus

Sl. No.	Latin name	Common name	Tospovirus	Major crops	Reference
1	*Frankliniella occidentalis*	Western Flower thrips	TSWV, INSV GRSV, TCSV, CSNV	Tomato Groundnut, Letuce, Impatiens.	Gardner *et al.*, 1935; Bezera *et al.*, 1999; Allen and Broadbent, 1986; Cho *et al.*, 1988; De Angelis *et al.*, 1993; Wijkamp *et al.*, 1995;
2	*F. schultzei*	Common blossom or cotton bud thrips	TSWV, TCSV, GRSV	Tomato	Samuel *et al.*, 1930; Wijkamp *et al.*, 1995.
3	*F. fusca*	Tobacco thrips	TSWV	Tomato, Tobacco	Sakimura, 1963.
4	*F. intonsa*	Flower thrips	TSWV, TCSV, GRSV	Tomato, Groundnut	Wijkamp *et al.*, 1995
5	*Thrips tabaci*	Onion thrips	TSWV, IYSV	Onion, Irish	Linford, 1932; Pittman, 1927; Sakimura, 1963; Corter *et al.*, 1998.
6	*Thrips setosus*	No common name	TSWV	-	Kobatake *et al.*, 1984.
7	*T. palmi*	Melon thrips	WSMV, GBNV	Watermelon, Groundnut	Iwaki *et al.*, 1984; Yeh *et al.*, 1992; Vijaya Lakshmi *et al.*, 1995.
8	*Scirtothrips dorsalis*	Chilli thrips	PBNV	Groundnut, Mungbean, Urdbean	Ghanekar *et al.*, 1979; Amin *et al.*, 1981.

TSWV = Tomato Spotted Wilt Virus
INSV = Impatiens Necrotic Spot Virus
GRSV = Groundnut Ring Spot Virus
TCSV = Tomato Chlorotic Spot Virus
IYSV = Irish Yellow Spot virus
WSMV = Watermelon Silver Mottle Virus
GBNV = Groundnut Bud Necrosis Virus
CSNV = Chrysanthemum Stem Necrosis Virus
PBNV = Peanut Bud Necrosis Virus

MATERIAL AND METHODS

III MATERIAL AND METHODS

The laboratory experiments during the present investigation were conducted in the insect proof glass house located at the Project Co-ordinating Unit (Sunflower), Regional Research Station, Bangalore and the field experiments in the plots at GKVK Campus of UAS, Bangalore. The material used and methods followed during the course of investigation are presented in this chapter.

3.1 Survey for the incidence of sunflower necrosis virus disease

The survey was under taken in the experimental plots, seed production plots and in the farmers fields, around Bangalore during different months of 1999-2000. In each of plot surveyed, about five rows were randomly selected and the per cent disease incidence was calculated as given below.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Information regarding places visited, genotype involved, stage of the crop, number of plants infected, total number of plants, name of the farmer, crop area and other information were also recorded during the course of survey. Disease diagnosis during the survey in the field was based on symptoms on leaves, petioles, stems and flower heads.

3.2 Inoculum

During the survey, good number of plants showing disease symptoms were collected from the field and brought to laboratory for further studies. While collecting the samples, information on symptoms produced by the disease like chlorotic spots on young leaves, systemic mosaic mottling and necrotic tissue were also recorded.

3.3 Transmission studies

3.3.1 Mechanical sap inoculation

The mechanical sap inoculation of the sunflower necrosis viral disease on the test plants was carried out using the following buffer.

Preparation of buffer

Potassium phosphate buffer (pH-7.0) :

Potassium dihydrogen phosphate	2.4 g
Dipotassium hydrogen orthophosphate	5.4 g
Thioglycerol	0.75 ml
Distilled water	1 L.
pH	7

(No adjustment of pH was needed)

* DW---> distilled water

3.3.2 Preparation of inoculum

Young tender leaves from the infected sunflower plants showing good chlorotic spots were collected, washed thoroughly in running tap water to remove dirt and blotter dried up. The leaf samples were ground in a clean, sterilized pestle and mortar kept in a container with ice cubes by adding each of the different chilled buffers separately 1 ml/gm of leaf tissue.

After thorough maceration, the pulp was squeezed through sterilized absorbent cotton. The resultant extract was used as "Standard inoculum" for sap inoculation.

3.3.3 Method of inoculation

An abrasive carborundum (600 mesh size) was dusted on the leaves of the test plants (sunflower var. Morden) using double layer muslin cloth (sunflower plants were raised from healthy seeds in 6 inches polythene bags containing sand, soil and compost in 2:1:2 ratio (W/W) and maintained in insect proof glasshouse). A small piece of sterilized absorbent cotton wool soaked in the standard inoculum was gently rubbed on the upper surface of the carborundum dusted leaves of the test plants. During inoculation, the leaves were supported from below with left hand palm to avoid any injury and to assure uniform pressure and spread of the inoculum. The inoculated leaves were washed few minutes after inoculation with a jet of sterile water from the squeeze bottle, to remove excess inoculum on the leaves. One set of plants

inoculated with different buffer, labeled separately and kept under observations for symptom expression up to 30 days in the insect proof glass house.

3.3.4 Maintenance of disease culture

The inoculated plants in the glass house showing characteristic symptoms were maintained on healthy seedlings of sunflower by periodic sap inoculations using standard buffer.

3.3.5 Determination of stage of susceptibility of sunflower plants to disease

In order to know the right stage of sunflower plants susceptible to disease, a set of sunflower plants var. Morden were raised in large numbers by staggered sowings. Inoculations were made by dusting carborundum (600 mesh) on cotyledonary leaves, first pair of true leaves, second pair of true leaves, second and third pair of true leaves and third and fourth pair of true leaves. The inoculated plants were labeled and kept under observation in the insect proof glass house for symptom expression till flower bud formation stage.

3.4 Seed transmission

Seeds from naturally infected plants in different genotypes of sunflower viz., Morden, CMS 234-B and RHA6D-1, MSFH-17, Sungene-85, PAC 1091, KBSH-1 were collected from the field. The collected seeds were kept for

30 days period to over come dormancy. Out of which, 150 seeds from each genotype were used for the study. Fifty seeds from each genotype were sown in separate seed pan filled with well mixed sand, soil and compost and maintained in insect proof glass house and replicated three times. The observations were recorded on germination percentage and number of plants showing symptoms till button formation stage and percentage incidence of disease, if any was worked out.

3.5 Insect transmission

In order to determine the transmissibility of virus by insect vectors, inoculations were made with thrips.

3.5.1 Thrips transmission

Test plants raised in nylon mesh cages were used in insect transmission experiments. Healthy thrips (*Thrips palmi* Karny) colonies were raised from adults collected from the field and maintained on sunflower plants by weekly transfer of active nymphs. Young sunflower leaves showing primary symptoms were kept in the petriplate. Along the rim of petriplate a thin layer of water is poured and about 20 to 30 nymphs were released on sunflower leaves showing characteristic symptoms of the disease using fine moist camel hair brush. The nymphs were allowed for Acquisition Access Period (AAP) of 3 days at room temperature. Nymphs fed on healthy leaves was served as check.

Twenty to twenty five nymphs of *T. palmi* were placed using a fine hair brush on seedlings of test plants which were raised in the nylon mesh cages. The normal movement of thrips was observed to ensure that injury had not occurred during the transfer. The plants were observed for 30 days to express the symptoms.

Jain *et al.* (2000) confirmed that the virus under study belongs to "tospovirus" sero group, which gave strong reaction to antisera of Water melon Silver Mottle Virus (WSMV) and Peanut Bud Necrosis Virus (PBNV). All tospoviruses are known to transmit by thrips. The virus under study belonging to tospovirus serogroup and now proved to be transmitted by thrips species, *T. palmi.*

3.6 Host range

Seventy plant species (which includes 40 crop plants and 30 weed species) belonging to different host families viz., Chenopodiaceae, Solanaceae, Leguminoceae, Cucurbitaceae, Malvaceae, Asteraceae, Pedaliaceae, Linaceae, Caricaceae, Euphorbiaceae, Cruciferae, Caesalpiniaceae, Convolvulaceae, Amaranthaceae, Verbenaceae, Labiatae, Rubiaceae, Commelinaceae, Boraginaceae, Cruciferae were selected for host range studies. Seeds of pulses and oils seed crops were collected from the pulses and oil seed schemes, UAS, GKVK, Bangalore - 65. Seeds of vegetable and other crops like cotton, papaya were collected from Department of Horticulture. Seeds of different weed species around GKVK Farm were collected. The different weed species were

identified by Balakrishna Gowda (Dept. of Botany) GKVK. The collected seeds were well dried and stored separately in polythene bags.

Transmission studies with the above 70 plant species have been carried out both by mechanical sap inoculation and by using thrips, separately and the procedure followed are described below :

3.6.1 Crop plants

3.6.1a Mechanical sap inoculation

The test plants of each species were raised from healthy seeds in 6 inches polythene bags containing soil, sand mixture 2:1 ratio (W/W) and maintained in insect proof glass house. A group of twenty five plants of each species/cultivar were sap inoculated following the method described in section 3.3.2 and 3.3.3 of this chapter. The leguminous plants were inoculated on cotyledonary leaves before the emergence of trifoliate leaves, species of *Nicotiana* were inoculated at 6-8 leaf stage and other test plants were inoculated on second and fourth fully expanded leaves. The plants were labeled and kept for symptom expression in glass house and observed up to 40 days. The plants which showed symptoms were checked for the presence of virus by back inoculation to sunflower test plants maintained in insect proof glass house.

3.6.1b Thrips transmission

The test plants of each species were raised from the healthy seeds in 6 inches polythene bags containing soil, sand mixture in 2:1 ratio (W/W) and maintained in insect proof glass house. Healthy thrips colonies were raised from adults collected from the field and maintained on sunflower plants by weekly transfer of active nymphs. Young sunflower leaves showing primary symptoms were kept in the petriplate. Along the rim of petriplate a thin layer of water is poured and about 20 to 30 nymphs were released on sunflower leaves showing characteristic symptoms of the disease using fine moist camel hair brush. The nymphs were allowed for AAP of $>$ 3 days at room temperature and nymphs fed on healthy leaves served as check.

Twenty to twenty five nymphs of *T. palmi* were placed with a fine hair brush on seedlings of test plants,(crop plants) were raised in the nylon mesh cages and were observed for symptom expression up to 40 days. Plant showing symptoms were checked for virus by back transmission to test plants maintained in insect proof glass house using healthy thrips by allowing them to feed on such plants.

3.6.2 Weeds

Weed plant species belonging to families Asteraceae, Euphorbiaceae, Amaranthaceae, Verbenaceae, Rubiaceae, Caesalpiniaceae, Labiatae, Commelinaceae, Malvaceae, Solanaceae, Boraginaceae, Convolulaceae,

Cruciferae were selected for weed host range studies both by mechanical sap inoculation and thrips transmission.

3.6.2a Mechanical sap inoculation

The test plants of each weed species were raised from healthy seeds in 6 inches polythene bags containing soil, sand mixture 2:1 ratio (W/W) and maintained in insect proof glass house. A group of twenty plants of each weed species (local) were sap inoculated following the method described in section 3.3.2 and 3.3.3 of this chapter. The test plants were inoculated on second and fourth fully expanded leaves. The plants were labeled and kept for symptom expression in glass house and observed up to 40 days. The plants which showed symptoms were checked for the presence of virus by back inoculation to test plants maintained in insect proof glass house.

3.6.2b Thrips transmission

Weed plants of each species from the families listed above were raised from healthy seeds in 6 inches polythene bags containing soil, sand mixture in 2:1 ratio (W/W) and maintained in insect proof glass house. Ten plants of each species or cultivar were kept inside the insect proof cages and thrips transmission was done by following the method described in section 3.6.1b and plants showing symptoms were checked for virus by back transmission to test plants maintained in insect proof glass house using healthy thrips by allowing them to feed on such plants.

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The experiments under investigations were conducted in the glass house located at Project Co-ordinating Unit, Regional Research Station, UAS, GKVK, Bangalore and also in the experimental plots at GKVK Campus. The results obtained from the experiments are presented in this chapter.

4.1 Survey for incidence of sunflower necrosis virus disease in and around Bangalore

Survey was undertaken for the incidence of sunflower necrosis viral disease in seed production plots, experimental plots and in farmers field in and around Bangalore. Information regarding places visited, incidence of disease, genotypes involved, month of sowing and other relevant information recorded during the survey are presented in the Table 4.

The results revealed that, the disease was prevalent in all the sunflower fields surveyed. In the seed production plots of Greentech, International Pvt. Ltd., Doddaballapur surveyed in June 1999, the per cent incidence among three genotypes ranged from 6.67 to 16.07. In seed production plot at Chikballapur the per cent incidence among three genotypes ranged from 7.57 to 10.35 during August 1999. In GKVK, experimental plots surveyed from June 1999 to February 2000, the per cent incidence was maximum (21.05 %) during January 2000.

Table 4 : Incidence of Sunflower necrosis virus disease in and around Bangalore

Sl. No.	Place	Variety/ Hybrid	Area (acre)	Number of Plants		Per cent Incidence	Month of sowing
				Observed	Infected		
1	Seed production plots of Green Tech. Pvt. Ltd., Doddaballapur	Hybrid	0.50	380	55	14.47	April (1999)
		Line - 1	0.50	280	45	16.07	
		Line - 2	1.00	600	40	6.67	
2	Seed production plots of Nath Seeds Pvt. Ltd., Chikkaballapur	Hybrid - 1	0.50	330	25	7.57	June (1999)
		Hybrid - 2	0.60	280	29	10.35	
		Hybrid - 3	0.50	170	15	8.82	
3	Experimental plots at GKVK campus UAS, Bangalore	Hybrid	0.25	190	40	21.05	April 99
		Morden	0.25	180	18	10.00	June 99
		Hybrid	0.50	130	10	7.69	June 99
		Morden	0.20	170	15	8.82	July 99
		Hybrid	0.20	200	15	7.50	Sept. 99
		Lines	0.20	175	9	5.14	Sept. 99
		Morden	0.40	200	10	5.00	Nov. 99
		Hybrid	0.50	212	10	4.72	Nov. 99
4	Experimental plots at Hebbal Campus UAS, Bangalore	A and B Lines	0.50	140	12	8.57	July 99
		F_1 inbreds	0.40	271	20	7.38	Sept. 99
		Hybrid	0.60	190	12	6.31	Oct. 99
		Morden	0.60	169	10	5.91	Oct. 99
		Hybrid	0.40	181	22	12.15	August 99
		A and B Lines	0.60	241	42	17.33	March 2000

At Hebbal Campus wherein seed production of A and B lines were taken up both during July 1999 and March 2000, the per cent incidence of the disease ranged from 8.57 to 17.33, respectively. Whereas, in the populations and hybrids, the per cent incidence ranged from 5.91 to 12.10.

The Table also revealed that the incidence of disease was more in crop sown in summer and kharif months and less during rabi season.

4.2 Symptomatology

Under field conditions

Various types of symptoms were observed during the survey under field conditions. The symptoms on infected sunflower plants initially appeared as chlorotic spots on leaves which of leaf lamina near midrib leading to twisting of leaves (Plate 1). The necrosis (black streak) extended through on side of the leaf lamina to the petiole and stem and finally terminated at the apical bud causing necrosis of tip.

In some infected plants, the tip of the growing parts became necrotic giving typical bud necrosis symptom, the plants failed to produce flowers and finally died (Plate 2). The flower buds were enlarge after flowering and became sterile. The infection reduced the internodal length leading to stunting of plants.

In some cases, the infection was found to become systemic resulting in mottling of various patterns, puckering of leaves, narrowing of leaves

Plate 1 : Sunflower plant infected with sunflower necrosis (SNV) under field conditions showing chlorotic spots on young leaves and necrosis of older leaves

Plate 2 : Sunflower plant infected with sunflower necrosis virus under field conditions showing necrosis of leaves and emerging flower bud

Plate 3 : Sunflower plant infected with sunflower necrosis virus under field conditions showing necrosis of calyx and corolla

Plate-1

Plate-2

Plate-3

and yellowing. Such plants remained stunted and produced small ear heads with necroted bracts (Calyx and Corolla) (Plate 3) twisting of the capitulum and necrosis on the back of the capitulum was found to be common. Further, infection could be seen at any stage of the plant.

During survey, few infected plants were seen to recover after infection producing normal ear heads which were smaller compared to healthy ones. Such plants were stunted with shorter internodal length in the middle of the stem when compared to healthy ones.

4.3 Transmission studies

4.3.1 Mechanical sap transmission

The standard inoculum prepared by using buffer was inoculated to healthy sunflower seedlings in green houses as described in material and methods chapter. The results of this experiment is presented in Table 5.

The standard inoculum prepared from potassium phosphate buffer (0.075 % thioglycerol) induced symptoms of chlorotic spots and necrosis of the tip on test plants. The percentage transmission ranged from 30.0 to 45.0, in Morden variety and two hybrids viz., KBSH-1 and PAC 1091.

Table 5 : Sap inoculation of Sunflower necrosis virus disease at two leaf stage

Sl. No.	Buffer	Variety/ hybrid	Number of Plants		Per cent transmission
			Inoculated	Infected	
1	Potassium Phosphate buffer (KH_2PO_4-0.01M K_2HPO_4-0.03M)	Morden	20	8	40.0
			20	8	40.0
			20	9	45.0
2	Potassium Phosphate buffer (KH_2PO_4-0.01M K_2HPO_4-0.03M)	KBSH-1	20	9	45.0
			20	6	30.0
			20	8	40.0
3	Potassium Phosphate buffer (KH_2PO_4-0.01M K_2HPO_4-0.03M)	PAC1091	20	8	40.0
			20	6	30.0
			20	8	40.0

4.3.2 Symptomatology

Under artificial inoculation

In the glass house on the inoculated plants the newly emerged leaves showed slight downward curling 3-4 days after inoculation (Plate 4). Such leaves developed chlorotic spots 8-10 days after inoculation (Plate 5). The chlorotic spots later turned necrotic, necrosis through petiole causing necrosis of the tip, 15-20 days after inoculation (Plate 6).

Some of the inoculated plant showed systemic mosaic mottling symptoms, 20-25 days after inoculation (Plate 7). In few other cases, plants initially showed mosaic mottling but later recovered to produce healthy leaves. Few inoculated plant which developed systemic symptoms later produced necrosis of bracts and calyx after button formation.

4.3.3 Effect of the stage of sunflower test plants on the mechanical sap transmission of the disease

It was observed under field conditions, the crop was susceptible to disease at all stages of its growth. In order to know the most susceptible stage for infection under artificial conditions, inoculations were made on test plants of different age groups (stages) and the results obtained were presented in Table 6.

The results (Table 6) revealed that the inoculations made on cotyledonary leaves failed to produce any symptoms. Inoculations made on first pair of true leaf stage and second pair of true leaf stage developed

Table 6 : Determination of right stage of sunflower test plants for the transmission of sunflower necrosis virus disease through sap

Sl. No.	Stage of test plants	Number of Plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
		Inoculated	Infected			
1	Cotyledonary leaf	(a) 20	0	0	-	
		(b) 20	0	0	-	
		(c) 20	0	0	-	
2	First pair of true leaf	(a) 20	8	40.0	8-10	Chlorotic spots
		(b) 20	8	40.0	15-20	Necrosis of the leaves and tip
		(c) 20	9	45.0		
3	Second pair of true leaf	(a) 20	8	40.0	8-10	Chlorotic spots
		(b) 20	9	45.0	15-20	Necrosis of the leaves and tip
		(c) 20	9	45.0		
4	Third pair of true leaf	(a) 20	5	25.0	10-15	Chlorotic spots
		(b) 20	5	25.0	20-25	Mosaic
		(c) 20	4	20.0		
5	Fourth pair of true leaf	(a) 20	5	25.0	10-15	Chlorotic spots
		(b) 20	4	20.0	20-25	Mosaic
		(c) 20	4	20.0		

Plate 4 : Sunflower cv. Morden showing curling of newly emerging leaves, 3-4 days after sap inoculation with sunflower necrosis virus

Plate 5 : Sunflower cv. Morden showing chlorotic spots on leaves, 8-10 days after sap inoculation with sunflower necrosis virus

Plate-4

Plate-5

Plate 6 : Sunflower cv. Morden showing necrosis of leaves and apical bud necrosis, 15-20 days after sap inoculation with sunflower necrosis virus

Plate 7 : Sunflower cv. Morden showing mosaic mottling symptom, 20-25 days after sap inoculation with sunflower necrosis virus

Plate-6

Plate-7

disease symptoms and percentage transmission ranged from 40.0 to 45.0. Inoculations made on the plants with third pair of true leaf stage and fourth pair of true leaf stage also produced disease symptoms. However, the percentage transmission was low ranging from 20.0 to 25.0

4.4 Seed transmission

Seeds collected from naturally infected sunflower genotypes viz., Morden, CMS 234-B, RHA 6D-1, KBSH-1, PAC 1091, MSFH-17 and Sungene-85 were sown in seed pan. The results obtained are presented in Table 7.

The results revealed that though good percentage of germination was obtained from the seeds collected from such field infected plants none of the seedlings developed disease symptoms even after button formation. This indicates the non-transmissible nature of the virus through seeds.

4.5 Insect transmission

4.5.1 Thrips transmission

Transmission studies conducted utilizing *T. palmi* colonies raised under laboratory conditions. Adults of the thrips species could able to transmit disease with three days of acquisition access period. However, it could not transmit the disease with shorter acquisition access periods of one and two days. The per cent transmission varied in three different sets. The

Table 7 : Seed transmission studies of the disease

Sl. No.	Variety/Hybrid	Number of Seeds		Per cent germination	Number of plants showing symptoms	Per cent incidence/ transmission
		Sown	Germinated			
1	Morden	(a) 50	45	90	-	-
		(b) 50	47	94	-	-
		(c) 50	47	94	-	-
2	CMS 234-B	(a) 50	38	76	-	-
		(b) 50	40	80	-	-
		(c) 50	42	84	-	-
3	RHA 6D-1	(a) 50	43	86	-	-
		(b) 50	43	86	-	-
		(c) 50	45	90	-	-
4	KBSH-1	(a) 50	45	90	-	-
		(b) 50	46	92	-	-
		(c) 50	43	86	-	-
5	MSFH-17	(a) 50	40	80	-	-
		(b) 50	42	84	-	-
		(c) 50	42	84	-	-
6	PAC 1091	(a) 50	42	84	-	-
		(b) 50	46	92	-	-
		(c) 50	46	92	-	-
7	Sungene - 85	(a) 50	46	92	-	-
		(b) 50	47	94	-	-
		(c) 50	47	94	-	-

per cent transmission ranged from 12.0 to 24.0, indicating the transmissible nature of the disease by *T. palmi* (Table 8) and three days of acquisition access period was found to be effective for thrips transmission of the isolate. The per cent transmission varied with variety, Morden (20 %), KBSH-1 (24 %), PAC 1091 (12 %) (Table.9).

Symptomatology

Upon inoculation by *Thrips palmi*

Typical symptoms of sunflower necrosis virus disease which include chlorotic spots or patches on one side of leaves (Plate 8), reduction in leaf size and stunting were observed on all infected seedlings, 10 to 15 days after inoculation (Plate 9). The chlorotic spots later turned necrotic (Plate 10), few plants produced chlorotic spots on leaf lamina(Plate 11). However, certain plants recovered to produce healthy leaves. Symptoms were observed on inoculated and on leaves which emerged soon after inoculation.

4.6 Host range

Seventy plant species belonging to different families viz., Cucurbitaceae, Chenopodiaceae, Malvaceae, Solanaceae, Leguminoceae, Asteraceae, Euphorbiaceae, Cruciferae, Caricaceae Linaceae, Caesalpiniaceae, Convolvulaceae, Amaranthaceae, Verbenaceae, Labiatae, Boraginaceae, Pedaliaceae, Rubiaceae, Commelinaceae were tested in order to find out the host range of sunflower necrosis virus both by

Table 8 : Thrips transmission of Sunflower necrosis virus disease

Sl. No.	Thrips species	Acquisition access period (AAP) (days)	Inoculation access period (IAP) (days)	Number of plants		Per cent transmission
				Inoculated	Infected	
Set I	*Thrips palmi*	1	5 - 7	25	0	0
		2	5 - 7	25	0	0
		3	5 - 7	25	6	24.0
Set II	*Thrips palmi*	1	5 - 7	25	0	0
		2	5 - 7	25	0	0
		3	5 - 7	25	4	16.0
Set III	*Thrips palmi*	1	5 - 7	25	0	0
		2	5 - 7	25	0	0
		3	5 - 7	25	3	12.0

Table 9 : Transmission of Sunflower necrosis virus disease through thrips on sunflower genotypes

Sl. No.	Thrips species	Variety/ hybrid	acquisition access period (days)	Inoculation access period (days)	Number of plants		Per cent transmission
					Inoculated	Infected	
1	*Thrips palmi*	Morden	1	5 - 7	25	0	0
			2	5 - 7	25	0	0
			3	5 - 7	25	5	20.0
2	*Thrips palmi*	KBSH-1	1	5 - 7	25	0	0
			2	5 - 7	25	0	0
			3	5 - 7	25	6	24.0
3	*Thrips palmi*	PAC 1091	1	5 - 7	25	0	0
			2	5 - 7	25	0	0
			3	5 - 7	25	3	12.0

Plate 8 : **Sunflower seedling showing chlorotic spots on leaves upon inoculation by *Thrips palmi* with SNV**

Plate 9 : **Sunflower seedling showing necrosis, twisting, downward curling, reduced growth of leaves upon inoculation by *T. palmi***

Plate-8

Plate-9

Plate 10 : Sunflower seedling showing necrosis of leaves upon inoculation by *T. palmi*

Plate 11 : Sunflower seedling showing chlorotic spots on leaf lamina upon inoculation with *T. palmi*

Plate-10

Plate-11

mechanical sap inoculation and Insect Vector Transmission (*T. palmi*) as described under the "Material and Methods". The results of this experiment are presented in Tables 10 to 13.

4.6.1 Crop plants

a) Mechanical sap inoculation

The results (Table 10) indicated that sunflower necrosis virus was able to infect the crop plants, groundnut, cowpea, soybean, horse gram, cucumber, watermelon, ridge guard when mechanical sap transmission was followed with standard extract of the infected leaves with potassium phosphate buffer. However, there were some differences with respect to production of symptoms on various host species and number of days taken for expression of various types of symptoms. The virus produced mosaic, chlorotic spots, turning to necrosis on sunflower leaves, 10-15 days after inoculation. The infected plants remained stunted.

On groundnut (cvs., JL-24 and K-134), the symptoms produced as mosaic followed by chlorosis on leaves, 15-20 days after mechanical sap inoculation (Plate 12). This was followed by systemic symptoms consisting of necrotic spots on subsequently emerging leaves (Plate 13) and such symptoms to develop takes 35-40 days after inoculation.

On soybean (cv. Hardy), the symptoms produced as mosaic mottling, followed by chlorotic streaks on leaves, 20 days after mechanical sap

inoculation (Plate14). This was followed by systemic symptoms consisting of round necrotic spots all along the leaf margin on subsequently emerging leaves (Plate 15) and such symptoms to develop takes 30-35 days after inoculation. Further, the infected plants remained stunted.

On cowpea (cvs., C-152 and Pusa komal), the symptoms produced as chlorosis on leaves, 8-10 days after mechanical sap inoculation (Plate 16). This was followed by thickening of leaf vein and there was reduction in leaf size, such symptoms to develop takes 15-20 days after inoculation (Plate 17).

On horse gram (cvs,. BGM 1 and Local variety) the symptoms produced as mild mosaic mottling, 8-10 days after mechanical sap inoculation. This was followed by reduced leaf size of infected plant and vein thickening, curling (Plate 18), there was drying of leaves and stunted growth of plants leading to tip necrosis after inoculation and such symptoms to develop takes 10-15 days.

On cucumber (cv. Green Long), the causal agent infects by producing mosaic and chlorotic spots four days after mechanical inoculation (Plate 19). This was followed by narrowing of leaves, stunted growth and there was drying of tip of the plant (Plate 20) resulting in apical necrosis of the infected plants and such symptoms to develop takes 5-6 days after inoculation.

Table 10 : Host range of sunflower necrosis virus disease through mechanical sap inoculation – crop plants

Sl. No.	Crop plant species	Family	Cvs.	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
				Inoculated	Infected			
1	*Arachis hypogaea* L.	Leguminoceae (Fabaceae)	JL-24, K-134	20	2	10	35-40	Mosaic, chlorosis and necrotic spots along the margin of leaf.
2	*Glycine max* L. Merr.	Leguminoceae (Fabaceae)	Hardy	20	4	20	30-35	Mosaic, mottling, chlorotic, streaks, necrotic spots all along the margin yellowing, reduced size of leaf.
3	*Brassica oleraceae* var capitata L.	Cruciferae (Brasicaceae)	MCW-17	20	0	-	-	-
4	*Carthamus tinctorious* L.	Asteraceae	Local	20	0	-	-	-
5	*Ricinus cummunis* L.	Euphorbiaceae	Aruna	15	0	-	-	-
6	*Sesamum indicum* L.	Pedaliaceae	Kanakapur a local	15	0	-	-	-
7	*Helianthus annuus* L.	Asteraceae	Morden	15	6	40	10-15	Chlorotic spots, mosaic, necrosis of leaves.
8	*Cajanus cajan* L. Hutch	Leguminoceae	TTB-7	20	0	-	-	-
9	*Pisum sativum* L.	Leguminoceae	Local	10	0	-	-	-
10	*Vigna unguiculata* L.	Leguminoceae	C-152, Pusa komal	20	4	20	15-20	Chlorosis on leaves and mosaic, vein thickening, reduced leaf size.
11	*Vigna mungo* L. Hepper	Leguminoceae	Local	20	0	-	-	-
12	*Vigna radiata* L. Wilegek	Leguminoceae	Local	20	0	-	-	-
13	*Phaseolus vulgaris* L.	Leguminoceae	Arka komal	20	0	-	-	-

Table 10 : (Contd….)

Sl. No.	Crop plant species	Family	Variety	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
				Inoculated	Infected			
14	*Dolichos lablab* L.	Leguminoceae	Local	20	0	-	-	-
15	*Macrotyloma uniflorum* L. syn. *Dolichos biflorus* L.	Leguminoceae	BGM-1, Local	20	3	15	15-20	Mosaic, mottling, drying of leaves, necrosis.
16	*Phaseolus aureus* L.	Leguminoceae	Local	20	0	-	-	-
17	*Phaseolus lunatus* L.	Leguminoceae	Local	20	0	-	-	-
18	*Cucumis sativus* L.	Cucurbitaceae	Green Long	15	3	20	5-6	Mosaic, chloritic spots, narrowing of leaves, drying of tip.
19	*Cucumis melo* L.	Cucurbitaceae	Arkajeet	15	0	-	-	-
20	*Citrullus lanatus* Thumb	Cucurbitaceae	Arkamanik	15	4	26.66	7-8	Mild mosaic, chlorosis, drying of tip.
21	*Cucurbita moscheta* Duch Expior	Cucurbitaceae	Arkasuryamukhi	15	0	-	-	-
22	*Luffa acutangufolia* Roxb.	Cucurbitaceae	Local	15	3	20	8-10	Mosaic and chlorotic spots.
23	*Lagenaria siceraria* Molina	Cucurbitaceae	Arkabahan	15	0	-	-	-
24	*Momordica charantia* L.	Cucurbitaceae	Local	15	0	-	-	-
25	*Benincasa hispida* (Thumb) Cogn.	Cucurbitaceae	Local	15	0	-	-	-
26	*Rhapanus sativus* L.	Cruciferae	Local	15	0	-	-	-

Plate 12 : Groundnut (cvs., JL-24 and K-134) showing mosaic followed by chlorisis on leaves, 15-20 days after sap inoculation with SNV

Plate 13 : Groundnut (cvs. JL-24 and K-134) spots along the leaf margin on emerging leaves, 35-40 days after sap inoculation with SNV

Plate-12

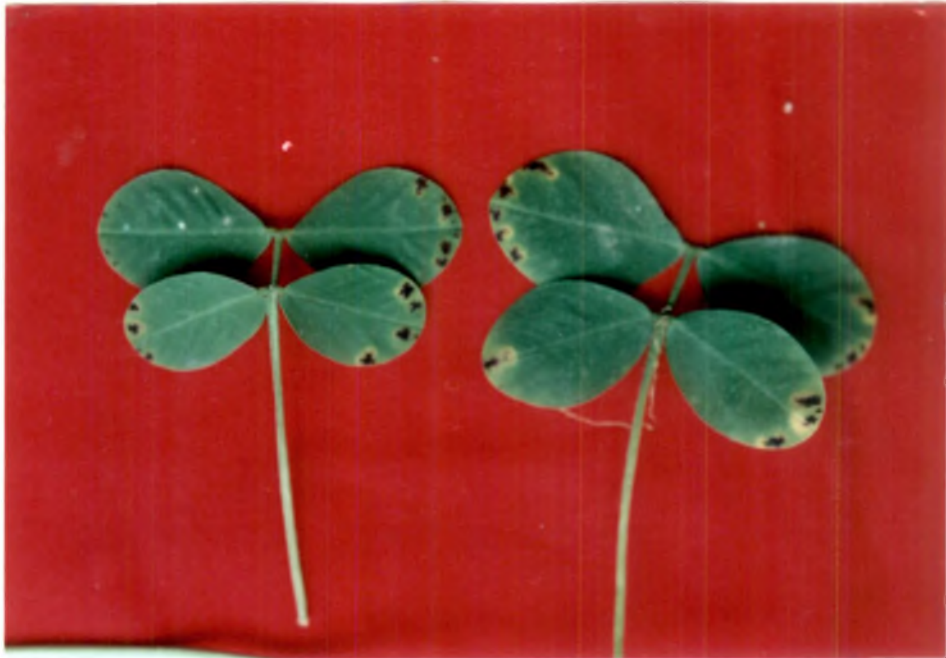

Plate-13

Plate 14 : Soybean (cv. Hardy) showing mosaic, chlorotic streaks on leaves, 20 days after sap inoculation with SNV

Plate 15 : Soybean (cv. Hardy) showing systemic necrotic spots all along the emerging leaves, 30-35 days after sap inoculation with SNV

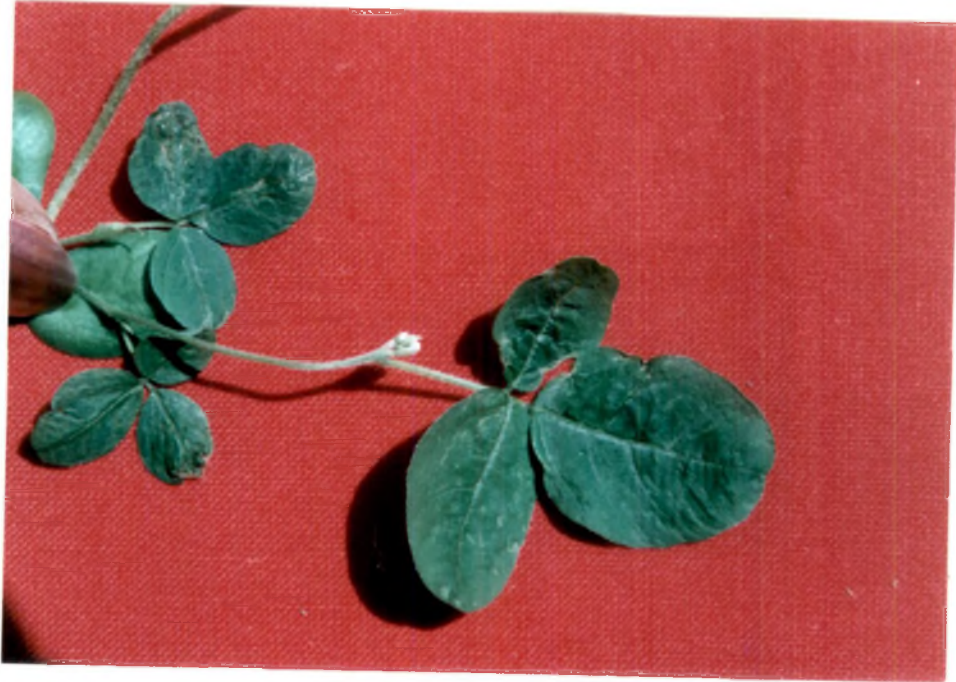

Plate-14

Plate-15

Plate 16 : Cowpea (cvs., C-152 and Pusa Komal) showing chlorosis on leaves, 8-10 days after sap inoculation with SNV

Plate 17 : Cowpea (cvs., C-152 and Pusa Komal) showing thickenign of leaf vein and reduced leaf size, 15-20 days after sap inoculation with SNV

Plate-16

Plate-17

Plate 18 : Horse gram (cvs., BGM 1 and Local variety) showing mild mosaic mottling, 8-10 days after sap inoculation with SNV

Plate-18

Plate 19 : Cucumber (cv. Green Long) showing mosaic and chlorotic spots on leaves 4 days after sap inoculation with SNV

Plate 20 : Cucumber (cv. Green Long) showing apical necrosis of plants 5-6 days after sap inoculation with SNV

Plate-19

Plate-20

Plate 21 : Water melon (cv. Arka Manik) showing necrosis of tip of terminal buds, 7-8 days after sap inoculation with SNV

Plate 22 : Ridge gourd (Local variety) showing mosaic symptom on leaves, 8-10 days after sap inoculation with SNV

Plate-21

Plate-22

On watermelon (cv. Arkamanik), the symptoms produced as mild mosaic followed by chlorosis on leaves, 5-6 days after mechanical sap inoculation. This was followed by systemic symptom with necrosis of tip of terminal buds (Plate 21) and such symptoms to develop takes 7-8 days after inoculation.

On ridge gourd (Local variety), the symptoms produced were mosaic followed by chlorosis, 8-10 days after mechanical sap inoculation (Plate 22). The infected plants remained stunted.

b) Insect vector transmission

Attempts were also made to transmit the virus through the insect vector (*T. palmi*) as described in material and methods. The results of insect vector transmission of virus from sunflower to different crop plants are presented in Table 11, which indicated that on several repetitions it was confirmed that thrips can transmit the sunflower necrosis virus from sunflower to sunflower, cucumber, watermelon, cowpea, groundnut, soybean, field bean, horse gram (with the acquisition access period of more than 3 days and inoculation access period of 5-7 days).

On groundnut (cvs., JL-24 and K-134), the symptoms produced as systemic necrotic spots on fresh emerging leaves (Plate 23) and such symptoms to develop takes 40-45 days after inoculation access period (IAP) and such plants remained stunted.

On soybean (cv. Hardy), the symptoms produced as mosaic mottling on leaves, 10-15 days after inoculation access period followed by chlorotic streaks on leaves, and such symptoms to develop takes 20-25 days after inoculation access period and infected plants remained stunted (Plate 24).

On cowpea (cvs., C-152 and Pusa komal), the symptoms produced were mosaic followed by curling on leaves, 10-15 days after inoculation access period (Plate 25). This was followed by systemic necrotic spots on leaves and such symptoms to develop takes 20-25 days after inoculation and infected plants remained stunted.

On filed bean (Local variety) the symptoms produced as systemic minute to small necrotic spots on leaves (Plate 26) followed by reduced leaf size, such symptoms to develop takes 25 days after inoculation access period and there was stunted growth of plants.

On horse gram (cvs., BGM 1 and Local variety) the symptoms produced as mosaic mottling 8-10 days after sap inoculation. This was followed by reduced leaf size of the infected plant and vein thickening, curling, 25-30 days after IAP (Plate 27).

On cucumber (cv. Green Long), the symptoms produced as mosaic followed by chlorotic spots on leaves, 10-15 days after inoculation access period. This was followed by necrotic spots on leaves drying of leaves and

Table 11 : Host range of sunflower necrosis disease through thrips vector (*Thrips palmi*) – crop plants

Sl. No.	Crop species	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
		Inoculated	Infected			
1	*Arachis hypogaea* L.	12	2	16.66	40-45	Necrotic spots on leaves
2	*Glycine max* L. Merr.	20	4	20.00	20-25	Mosaic, mottling, chlorotic streaks,
3	*Brassica oleraceae* var capitata L.	12	-	-	-	-
4	*Carthamus tinctorious* L.	10	-	-	-	-
5	*Ricinus cummunis* L.	10	-	-	-	-
6	*Sesamum indicum* L.	20	-	-	-	-
7	*Helianthus annuus* L.	25	6	24.0	10-15	Chlorotic spots, or patches necrotics on leaves.
8	*Cajanus cajan* L. Hutch	15	-	-	-	-
9	*Pisum sativum* L.	10	-	-	-	-
10	*Vigna unguiculata* L.	15	4	26.66	20-25	Mosaic, curling, necrosis on leaves
11	*Vigna mungo* L. Hepper	10	-	-	-	-
12	*Vigna radiata* L. Wilegek	10	-	-	-	-
13	*Dolichos lablab* L.	10	2	20	25-30	Necrotic spots, Reduced leaf size
14	Macrotylama uniflorum L. Syn. (Dolicos biflorus L.)	12	3	25	25	Mosaic, mottling, drying of leaves,
15	*Phaseolus aureus* L.	10	-	-	-	-

Table 11 : (Contd....)

Sl. No.	Crop species	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
		Inoculated	Infected			
16	*Phaseolus vulgaris* L.	10	-	-	-	-
17	*Phaseolus lunatus* L.	10	-	-	-	-
18	*Cucumis sativus* L.	16	4	25	15-20	Mosaic, necrotic spots on leaves drying of leaves
19	*Cucurbits moschata*	8	-	-	-	-
20	*Cucumis melo* L.	10	-	-	-	-
21	*Citrullus lanatus* (Thumb)	18	6	33.33	15-20	Drying of tips
22	*Luffa acutangafolia* Roxb.	10	-	-	-	-
23	*Lagenaria sicenaria* Molina	10	-	-	-	-
24	*Momordica charnatia* L.	10	-	-	-	-
25	*Rhapanus sativus* L.	10	-	-	-	-
26	*Capsicum annuum* L.	20	-	-	-	-
27	*Lycopersicon esculentum* Mill	20	-	-	-	-
28	*Nicotiana glutinosa* L.	10	-	-	-	-
29	*Nicotiana tabacum* var. Samsun	10	-	-	-	-
30	*Nicotiana benthamina* L.	10	-	-	-	-

Table 11 : (Contd....)

Sl. No.	Crop species	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
		Inoculated	Infected			
31	*Gossypium hirsutum*	10	0	-	-	-
32	*Malva neglecta*	5	0	-	-	-
33	*Chenopodium amaranticolor* Coste & Reyn	6	0	-	-	-
34	*Carica papaya* L.	5	0	-	-	-
35	*Solanum melongina* L.	5	0	-	-	-
36	*Guizotia abyssinica* L.	10	0	-	-	-
37	*Abelmosuchus exculantus* L.	5	0	-	-	-
38	*Cyamopsis tetragonoloba* L.	5	0	-	-	-
39	*Mentha arvensis*	5	0	-	-	-
40	*Benincasa hispide* Thumb Cogn.	8	0	-	-	-

Plate 23 : Groundnut (cvs., JL-24 and K-134) showing systemic necrotic spots on fresh emerging leaves, 40-45 days after inoculation access period (IAP) by *T. palmi* with SNV

Plate 24 : Soybean (cv. Hardy) showing mosaic mottling upon inoculation by *T. palmi* with SNV

Plate-23

Plate-24

Plate 25 : Cowpea (cvs., C-152 and Pusa Komal) showing necrotic spots, curling of leaves upon inoculation by *T. palmi* with SNV

Plate 26 : Field bean (Local variety) showing minute to small necrotic spots, reduced leaf size upon inoculation by *T. palmi* with SNV

Plate -25

Plate -26

Plate 27 : Horse gram (cvs., BGM 1 and Local variety) showing mild mosaic, vein thickening, curling of leaves upon inoculation by *T. palmi* with SNV

Plate 28 : Cucumber (cv. Green Long) showing, mosaic, chlorotic spots, 10-15 days after IAP followed by necrotic spots on leaves, 15-20 days after IAP by *T. palmi* with SNV

Plate -27

Plate -28

Plate 29 : Water melon (cv. Arka Manik) showing necrosis of tip of terminal buds upon inoculation by *T. palmi*

Plate-29

terminal shoot and such symptoms to develop takes 15-20 days after inoculation (Plate 28). The infected plants remained stunted.

On water melon (cv. Arkamanik), the symptoms produced were systemic necrosis of tip of terminal buds 15-20 days after inoculation access period (Plate 29).

4.6.2 Weeds plant species

Apart from crop plants different weed hosts (belonging to different families) were also tested for virus transmission both by mechanical sap inoculation and insect vector transmission (*T. palmi*).

a) Mechanical sap inoculation

The results (Table 12) revealed that sunflower necrosis virus was able to infect only two weed host species through mechanical sap inoculation namely *Euphorbia geniculata* L. and *Galinsoga parviflora* Cau.

On *Euphorbia geniculata* L. the symptoms produced as mosaic followed by chlorotic spots on leaves, 10-15 days after mechanical sap inoculation. This was followed by curling and drying of leaves (Plate 30) and such symptoms to develop takes 20-25 days after inoculation.

Table 12 : Host range of sunflower necrosis virus disease through mechanical sap inoculation – weed plant species

Sl. No.	Weed plant species	Family	Variety	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
				Inoculated	Infected			
1	*Ageratum conyzoyides* L.	Asteraceae	-	15	0	-	-	-
2	*Bidens biternata* L.	Asteraceae	-	15	0	-	-	-
3	*Tridax procumbens* L.	Asteraceae	-	15	0	-	-	-
4	*Lagascea mollis* Cau.	Asteraceae	-	15	0	-	-	-
5	*Acanthospermum hispidum* DC	Asteraceae	-	15	0	-	-	-
6	*Parthenium hysterophorus* Linn.	Asteraceae	-	15	0	-	-	-
7	*Spilanthus calva* DC	Asteraceae	-	15	0	-	-	-
8	*Euphorbia hirta* Linn.	Euphorbiaceae	-	15	0	-	-	-
9	*Euphorbia geniculata* Linn.	Euphorbiaceae	-	15	4	26.66	20-25	Mosaic, chlorotic spots, yellowing curling of leaves and drying of leaves
10	*Borreria ocymoides*	Rubiaceae	-	15	0	-	-	-
11	*Galinsoga parviflora* Cou.	Asteraceae	-	15	2	13.33	20-25	Mild mosaic, darkgreening, vein thickening and folding of leaves
12	*Ocimum americanum* L.	Labiatae	-	15	0	-	-	-
13	*Malvastrum coromandelianum* L.	Malvasceae	-	15	0	-	-	-
14	*Datura stramonium* Linn.	Solanaceae	-	15	0	-	-	-
15	*Xanthium stromanium* Linn.	Asteraceae	-	15	0	-	-	-
16	*Cassia hirsuta* Linn.	Caesalpiniaceae	-	15	0	-	-	-

Table 12 : (Contd….)

Sl. No.	Weed plant species	Family	Variety	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
				Inoculated	Infected			
17	*Sonchus oleraceus*	Asteraceae	-	15	0	-	-	-
18	*Syndrella nodiflora*	Asteraceae	-	15	0	-	-	-
19	*Crotalaria striata* Linn.	Fabaceae	-	15	0	-	-	-
20	*Ipomoea aquatica*	Convolvulaceae	-	15	0	-	-	-
21	*Achyranthus aspera* L.	Amaranthaceae	-	15	0	-	-	-
22	*Chromolana odorata*	Asteraceae	-	15	0	-	-	-
23	*Stachytarpheta jamaicensis*	Verbenaceae	-	15	0	-	-	-
24	*Amaranthus viridis* Linn.	Amaranthaceae	-	12	0	-	-	-
25	*Commalina bengalensis*	Commelinaceae	-	15	0	-	-	-
26	*Trichodesma zeylanica* L.	Boraginaceae	-	15	0	-	-	-
27	*Brassica nigra*	Cruiferace	-	15	0	-	-	-
28	*Eupatorium purpureum* Linn.	Asteraceae	-	15	0	-	-	-
29	*Oxalis europaca*	Asteraceae	-	12	0	-	-	-
30	*Solanum nigrum* Linn.	Solanaceae	-	10	0	-	-	-

Plate 30 : ***Euphorbia geniculata*** **showing mosaic, chlorotic spots 10-15 days after inoculation followed by curling of leaves, 20-25 days after sap inoculation with SNV**

Plate -30

Plate 31 : ***Galinsoga parviflora*** **showing mild mosaic followed by dark greening, 10-15 days after sap inoculation with SNV**

Plate 32 : ***Galinsoga parviflora*** **showing puckering of leaves, 20-25 days after sap inoculation with SNV**

Plate -31

On *Galinosoga parviflora* Cau., the symptoms produced as mild mosaic followed by dark greening and vein thickening of leaves, 10-15 days after mechanical sap inoculation (Plate 31). This was followed by puckering of leaves (Plate 32) and such symptoms to develop takes 20-25 days after inoculation. There was stunted growth of plants compared to healthy plants.

b) Thrips transmission

Attempts were also made to transmit the virus through insect vector, thrips (*T.palmi*) as described in "Material and Methods". The results of insect vector transmission of virus form sunflower to weed host plants are presented in Table 13. It revealed that none of the weed host plants were produced disease symptom even after 50 days of inoculation access period by *Thrips palmi*, even with several repetitions during the investigations.

Further, upon back inoculation both from infected crop plants and weed host plants as described above, to healthy sunflower test plant seedlings, produced typical disease symptoms as described on sunflower, elsewhere.

The causal agent failed to infect other genotypes of different families both by mechanical sap inoculation and insect vector transmission.

Table 13 : Host range of Sunflower necrosis virus disease through thrips vector (*Thrips palmi*) – weed plant species

Sl. No.	Weed plant	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
		Inoculated	Infected			
1	*Agerantum conyzoides*L.	20	0	-	-	-
2	*Bidens biternata* L.	10	0	-	-	-
3	*Tridax procumbens* L.	20	0	-	-	-
4	*Lagascea mollis* Cau.	10	0	-	-	-
5	*Acanthospermum hispidum* DC	10	0	-	-	-
6	*Parthenium hysterophorus* L.	10	0	-	-	-
7	*Spilanthus calva* DC	10	0	-	-	-
8	*Euphorbia hirta* L.	12	0	-	-	-
9	*Euphorbia geniculata*	20	0	-	-	-
10	*Borreria ocymoides*	10	0	-	-	-
11	*Galinsoga parviflora* Cau.	20	0	-	-	-
12	*Ocimum americanum* L.	8	0	-	-	-
13	*Malvastrum coromandelianum* L.	8	0	-	-	-
14	*Datura stramonium*	20	0	-	-	-
15	*Xanthium stromarium*	8	0	-	-	-
16	*Cassia hirsuta*	10	0	-	-	-
17	*Sonchus oleraceus*	15	0	-	-	-

Table 13 : (Contd....)

Sl. No.	Weed plant species	Number of plants		Per cent transmission	Time taken for symptom expression (days)	Symptoms observed
		Inoculated	Infected			
18	*Syndrella nodiflora*	15	0	-	-	-
19	*Crotolaria striata*	8	0	-	-	-
20	*Ipomoea aquatica*	10	0	-	-	-
21	*Achyranthus aspera* L.	10	0	-	-	-
22	*Chromolaena odorata*	10	0	-	-	-
23	*Stachytarpheta jamaicensis*	20	0	-	-	-
24	*Amaranthus viridis*	20	0	-	-	-
25	*Commelina bengalensis*	10	0	-	-	-
26	*Trichodesma zeylania*	10	0	-	-	-
27	*Brassica nigra*	10	0	-	-	-
28	*Euphotorium purpureum*	10	0	-	-	-
29	*Oxalis europaea*	10	0	-	-	-
30	*Solanum nigrum*	8	0	-	-	-

A comparison of symptoms produced by the causal agent (SNV) on various crop plants and weed species by following mechanical sap and insect vector, *Thrips palmi* was made and summarised below :

On sunflower the virus produced mosaic symptoms 8-10 days after mechanical sap inoculation, followed by chlorotic spots turning to necrosis on leaves 10-15 days after inoculation. Whereas, the virus produced more or less similar types of symptoms 8-10 days of inoculation access period by thrips on sunflower.

On groundnut (cvs., JL-24 and K-134) the virus produced mosaic symptoms followed by chlorosis on leaves, 15-20 days after mechanical sap inoculation. This was followed by systemic symptoms consisting of necrotic spots on subsequently emerging leaves and such symptoms to develop takes 35-40 days after inoculation. Whereas, the virus produced only systemic necrotic spots of small to medium size on leaves 40-45 days after inoculation access period of insect vector thrips.

On soybean (cv. Hardy) the Sunflower Necrosis Virus (SNV) produced mosaic mottling followed by chlorotic streaks, 20 days after mechanical sap inoculation. This was followed by systemic symptoms consisting of round necrotic spots all along the leaf margin on subsequently emerging leaves and such symptoms to develop takes 30-35 days after inoculation. Whereas, the virus produced only mosaic mottling symptoms 20 days after inoculation access period of thrips,

followed by chlorotic streaks on leaves 20-25 days after inoculation. But there is no production of necrotic spots.

On cowpea (cvs., C-152 and Pusa komal), the virus produced symptoms as chlorosis on leaves and vein thickening, reduction in leaf size 15-20 days after mechanical sap inoculation. Whereas, the virus produced mosaic followed by chlorosis 10-15 days after inoculation access period (IAP). This was followed by systemic symptoms of necrosis on leaves 20-25 days after inoculation.

On water melon (cv. Arka Manik) the virus produced mild mosaic symptoms followed by chlorosis on leaves 5-6 days after mechanical sap inoculation. This was followed by drying of tip of terminal bud 7-8 days after inoculation. Whereas, the virus produced only systemic necrosis of tip of terminal bud 15-20 days after inoculation access period.

On cucumber (cv. Green Long), the symptoms produced as mosaic followed by chlorotic spots on leaves 4 days after mechanical sap inoculation. This was followed by systemic drying of tip resulting into necrosis 5-6 days after inoculation. Whereas, the causal agent produced mosaic symptoms 10-15 days after inoculation access period of thrips and this was followed by systemic necrotic spots on newly emerging leaves, 15-20 days after inoculation.

On horse gram (cvs., BGM-1 and Local variety) the symptoms produced as mosiac mottling on leaves 8-10 days after mechanical sap

inoculation. This was followed by drying of leaves 10-15 days after inoculation. Similar types of symptoms were produced by the virus, 25-30 days after IAP.

On field bean (Local variety), causal agent was transmitted only by insect vector thrips and symptoms produced as minute to small systemic necrotic spots on leaves.

On ridge gourd (Local), the causal agent was transmitted only by mechanical sap inoculation by producing mosaic symptoms on leaves. This was followed by chlorotic spots on leaves, 8-10 days after inoculation.

Among different weed host plants, the sunflower necrosis virus infects on two weed hosts, only through mechanical sap inoculation. On *Euphorbia geniculata* the virus transmitted only by mechanical sap inoculation, the symptoms produced were mosaic and chlorotic spots on leaves, 10-15 days after inoculation. This was followed by curling and drying of leaves, 20-25 days after inoculation.

On *Galinsoga parviflora,* the symptoms produced as mild mosaic followed by dark greening and vein thickening of leaves, 10-15 days after mechanical sap inoculation. This was followed by puckering of leaves and such symptoms to develop takes 20-25 days after inoculation.

However, none of the weed host plants took infection and produced disease symptoms even after 50 days of inoculation access period when they were inoculated with thrips vector, even with several repetitions during the investigations.

DISCUSSION

V DISCUSSION

Sunflower (*Helianthus annuus* L.) is an important oil seed crop in India and has occupied a large area under cultivation in Karnataka. It is reported to be susceptible to several diseases caused by various agents. The most important ones being Alternaria leaf spot (*Alternaria helianthi*), rust (*Puccinia helianthi*) and downey mildew (*Plasmopara halstedii*), (Kolte, 1985).

Several virus and virus like diseases viz., sunflower mosaic, rugose mosaic, yellow ring mosaic, yellow spot, chlorotic leaf mosaic, greening, cucumber mosaic and mycoplasma like organism (MLO) (strain of tomato big bud, stolber, aster yellows and phyllody have been reported to infect sunflower (Nagaraju, 1995).

Very recently, a new virus disease on sunflower showing necrotic symptoms followed by various types of mosaic mottling, systemic necrosis and necrosis of other plant parts, leading to severe yield losses have been reported to occur around Bangalore (Anon, 1997) and further, the disease has been resulted to be more because of its fast spreading nature and severity, It is prevalent in most of the sunflower growing areas of Andhra Pradesh, Tamil Nadu and Maharashtra causing total loss of the crop (Anon, 2000).

However, the causal agent has been successfully transmitted through mechanical sap inoculation by Anil Kumar (1999) from sunflower to sunflower

and also through insect vector (*Thrips palmi*) by Shivasharanayya (2000), very recently.

In the present investigation, an attempt has been made to establish the transmission through several means, host range with more emphasis on the possible weed hosts of the virus in perpetuations. The result of these studies are precisely discussed in this chapter.

The results on the survey for the prevalence of the sunflower necrosis virus disease in all the sunflower fields visited during the course of investigation both in farmers field, seed production plots, fields of private firms and also in the experimental plots at GKVK and Hebbal Campuses around Bangalore resulted the prevalence of the disease in varying incidence in different sowing dates. Further, the disease was found to occur in all the seasons with varying incidence (%) around Bangalore. The maximum incidence was observed during summer months (April sown crop) (21.05%), whereas, it was in between on the crops sown in kharif (10 %) and least during rabi (4.72 %). Nagaraju *et al.* (1998) also recorded and reported the prevalence of high incidence of the disease up to 35 per cent during summer months, Anil Kumar (1999) and Shivasharanayya (2000) recorded and reported maximum incidence 21.0 and 21.1 per cent respectively during summer months.

Further, they (Anil Kumar, 1999; Shivasharanayya, 2000; Anon, 2000) also reported that the incidence of disease decreased after the on set of rains, during kharif months, decreasing gradually in rabi months. Similar results

were obtained in the present investigations, which might be very well related to the increased vector activity in the dry spell months, as was reported by Shivasharanayya(2000).

Halakeri (1999) reported that the disease could be seen at all stages of the crop growth. The incidence ranged from 3 to 70 per cent on this crop in Dharwad. Incidence and severity was higher in the summer months as compared to kharif and rabi season.

Symptomatological observations under field conditions revealed that the disease symptoms appeared as sudden necrosis of part of leaf lamina, the necrosis extending as black steak through the petiole and to the terminal shoot. The infected plant and flower buds were partially, twisted which later became sterile. In some plants, systemic symptoms developed after initial necrosis as various types of mosaic mottling, puckering, twisting of leaves, narrowing and yellowing. Such systemically infected plants remain stunted. In some plants after initial necrosis and subsequently systemic mosaic symptoms, lead to necrosis of terminal shoot and buds. Some plants were found to recover after initial symptom of necrosis which are shorter compared to healthy ones. Such plants were initially stunted due to reduced internodal length and later became normal producing flowers and seeds, showing recovery from the infection by the virus.

Further, the infection could be seen at all the stages of the crop growth. The symptoms observed were similar to the ones reported on this crop from

Bangalore (Anon, 1997; 1998; 2000; Nagaraju *et al.*, 1998; Anil Kumar, 1999 and Shivasharanayya, 2000).

With the detailed information available on the mechanical sap transmission [Anil Kumar (1999) and Shivasharanayya (2000),] the sap transmission of the disease was attempted with potassium phosphate buffer (0.05 M) (KH_2PO_4 - 0.01 M and K_2HPO_4 - 0.03 M) with 0.075 per cent Thioglycerol as anti-inhibitor which resulted in the highest percentage transmission (45.0) of the disease on different cultivars viz., Morden, KBSH-1 and PAC 1091.

Similar results with successful transmission of tomato spotted wilt virus (TSWV) through sap using 0.05 M potassium phosphate buffer (pH 7.0) with 0.02 M 2-mercaptoethanol as anti-inhibitor was reported by Prasad Rao *et al.* (1980). Hemalatha (1999) reported that the tomato necrotic spot tospovirus (TNSV) could be successfully transmitted by mechanical sap inoculation using 0.05 M potassium phosphate buffer (pH 7.0) containing 0.02 M 2-mercaptoethanol. Mechanical sap transmission of this virus has also been reported successfully by Anil Kumar (1999), Shivasharanayya (2000) and Halakeri (1999).

The symptoms on seedlings of sunflower cv. Morden upon mechanical sap inoculation, initially appeared as downward curling of freshly emerged leaves, 3-4 days after inoculation. These leaves showed chlorotic spots,

8-10 days after inoculation, which turned necrotic. This necrosis extending to the terminal bud through petiole causing necrosis of apical bud, 15-20 days after inoculation. In some of the inoculated and infected plants, systemic mosaic mottling symptoms were observed, 20-25 days after inoculation. Some inoculated and infected plants also produced necrosis of calyx and bracts after button formation.

The symptoms observed were similar to the virus reported on this crop by Anil Kumar (1999) and Shivasharanayya (2000) from Bangalore under laboratory conditions and also under field conditions (Nagaraju *et al.*,1998).

The virus under investigation with respect to the stage of susceptibility of the sunflower test plants revealed a high transmission percentage of (40.0 to 45.0 %), when seedlings with first and second pair of true leaves were used for inoculation than after development of subsequent pair of true leaves, wherein, the transmission percentage went on decreasing. However, no transmission was obtained when cotyledonary leaves were inoculated. Anil Kumar (1999) reported the similar type of results where in transmission percentage was 40.0 to 46.6% , when, first and second pair of true leaves were used for inoculation.

Thus the present study revealed that cotyledonary stage of seedlings was not right stage for sap inoculation. Whereas, the seedlings with first and second pair of true leaf stage were found to be the right stage for inoculations under glass house conditions.

Seed transmission studies using seeds collected from naturally infected plants of different sunflower genotypes gave negative results when tested under glass house conditions even up to pollination and flowering stage, indicating non-transmissible nature of the virus under study through seeds. Similar negative results with seed transmission of sunflower necrosis virus disease was reported by Anil Kumar (1999) and Halakeri (1999) and Shivasharanayya (2000).

The transmission studies with *T. palmi* gave positive results when a longer acquisition access period of three days was given. However, acquisition access period of less than three days gave no positive results even with several repetitions. The successful thrips transmission of sunflower necrosis virus (SNV) was also reported by Shivasharanayya (2000). However, because of the difficulty in maintenance of the thrips culture in laboratory, thrips were collected in large number using aspirator from field grown sunflower crop every time and used for transmission studies. The symptoms produced upon inoculation through thrips were similar to the symptoms reported to produce on sunflower under field conditions. (Nagaraju *et al.*, 1998; Anil Kumar, 1999; Halakeri, 1999 and Shivasharanayya, 2000) and also through thrips under laboratory conditions (Shivasharanayya, 2000).

This results also supplements the first record of successful thrips transmission of sunflower necrosis virus disease occurring on sunflower since the report of its occurrence during 1997 by Shivasharanayya (2000).

Jain *et al.* (2000) confirmed that the virus under study belongs to "tospovirus" sero group, which gave strong reaction to antisera of Water melon Silver Mottle Virus (WSMV) and Peanut Bud Necrosis Virus (PBNV). All tospoviruses are known to be transmitted by thrips. The virus under study belongs to tospovirus sero group and now proved to be transmitted by thrips species, *Thrips palmi* Karny.

Tomato Spotted Wilt Virus (TSWV) and Impatiens Necrotic Spot Virus (INSV) groups were more or less transmitted by *Frankliniella fusca* (Gardner *et al.*, 1935; Pitman, 1927; Mulder *et al.*, 1991; Sakimura, 1961). Peanut Bud Necrosis Virus (PBNV) and Water melon Silver Mottle Virus (WSMV) groups were transmitted only by *Thrips palmi*, whereas, Tomato Spotted Wilt Virus (TSWV) group may not be transmitted by *T. palmi* (Cho *et al.*, 1988; Mau *et al.*, 1991). Peanut Yellow Spot Virus (PYSV) was transmitted by *Scirtothrips dorsalis* (Amin *et al.*, 1981).

In the present investigation, yellow chlorotic spots and yellow patches were produced on young emerging leaves upon inoculation through thrips with 8-10 days of inoculation access period. In most of the seedlings, yellow chlorotic spots later turned necrotic, plants became stunted and vigour of the plants was reduced to a greater extent. Similar results have been reported by Shivasharanayya (2000).

In host range studies the causal agent was inoculated both by mechanical sap and insect vector to different crop plants and weed host species belonging

to families Chenopodiaceae, Cucurbitaceae, Asteraceae, Malvaceae, Solanaceae, Cruciferae, Caricaceae, Leguninoceae, Euphorbiaceae, Verbenaceae, Linaceae, Caesalpiniaceae, Convolvulaceae, Amaranthaceae, Boraginaceae, Pedaliaceae, Commelinaceae, Rubiaceae, Labiatae.

The present investigation on this aspect reveals that the Sunflower necrosis virus was able to infect the following crop plants viz., sunflower, groundnut, cowpea, soybean, watermelon, cucumber, horse gram, ridge guard and two weed host species *Euphorbia geniculata, Galinsoga parviflora* by mechanical sap inoculation.

On sunflower (cv. Morden and hybrids KHSH-1, PAC-1091), the virus produced mosaic symptoms followed by chlorotic spots, turning to necrosis on leaves, 10-15 days after inoculation.

On groundnut (cvs., JL-24 and K-134), the symptoms produced were mosaic followed by chlorosis on leaves, 15-20 days after mechanical sap inoculation. This was followed by systemic symptoms consisting of small to medium sized necrotic spots on subsequently emerging leaves and such symptoms to develop takes 35-40 days after inoculation.

On soybean (cv. Hardy), the symptoms produced as mosaic mottling, followed by chlorotic streaks on leaves, 20 days after mechanical sap inoculation. This was followed by systemic symptoms consisting of round

necrotic spots all along the leaf margin on subsequently emerging leaves and such symptoms to develop takes 30-35 days after inoculation. Further the infected plants remained stunted.

On cowpea (cvs., C-152 and Pusa komal), the symptoms produced as chlorosis on leaves, 8-10 days after mechanical sap inoculation. This was followed by thickening of leaf vein and there was reduction in leaf size of infected plant. Such symptoms to develop takes 15-20 days after inoculation.

On horse gram (cv., BGM-1 and Local variety), the symptoms produced are mosaic mottling, 8-10 days after mechanical sap inoculation. This was followed by reduced leaf size of infected plants, vein thickening, curling and there was drying of leaves and stunted growth of plants leading tip necrosis after inoculation and such symptoms to develop takes 10-15 days.

On cucumber (cv. Green Long), the causal agent infected by producing mosaic and chlorotic spots 4 days after mechanical sap inoculation. This was followed by narrowing of leaves stunted growth and there was drying of tip of the plant resulting in apical necrosis and such symptoms to develop takes 5-6 days after inoculation.

On water melon (cv. Arka Manik) the symptoms produced are mild mosaic followed by chlorosis on leaves 5-6 days after mechanical sap inoculation. This was followed by systemic symptoms with necrosis of tip of terminal buds and such symptoms to develop takes 7-8 day after inoculation.

On ridge gourd (Local variety) the symptoms produced as mosaic followed by chlorosis, 8-10 days after mechanical sap inoculation. The infected plants remained stunted.

On *Euphorbia geniculata*, symptom produced as mosaic followed by chlorotic spots on leaves, 10-15 days after mechanical sap inoculation. This was followed by curling and drying of leaves and such symptoms to develop takes 20-25 days after inoculation.

On *Galinsoga parviflora* the symptoms produced as mild mosaic followed by dark greening and vein thickening of leaves, 10-15 days after mechanical sap inoculation, This was followed by puckering of leaves and such symptoms to develop takes 20-25 days after inoculation. There was stunted growth of plants compared to healthy plants.

Host range/weed host of sunflower necrosis virus has not been done, as result reveals, so far, except Anil Kumar (1999) reported that out of 44 plants species tested, the pathogen infected only *Cucumus sativus* (cv. Green Long) producing chlorotic spots followed by necrosis of the leaves along margin, narrowing of leaves and stunting of plants. However, similar studies with other members of the tospovirus group (PBNV, WBNV etc.,) revealed that they could infect several host plants viz., tomato, cowpea, groundnut, red gram, soybean, watermelon, cucumber, squash , french bean, datura, nicotiana, horse gram, chilli, brinjal cluster bean so on by producing various types of symptoms.

Percentage of transmission with mechanical sap inoculation of sunflower necrosis virus was higher in case of sunflower (45.0%) as compared to other test host plants. The percentage transmission was 26.66 per cent in water melon and weed hosts *Euphorbia geniculata* followed by 20.00 per cent each in soybean, cowpea, cucumber, ridge gourd, 15.00 per cent in horse gram, 13.33 per cent in *Galinosa parviflora* and 10.00 per cent groundnut.

Anil Kumar (1999) reported the 46.6 per cent mechanical sap transmission on sunflower followed by cucumber 20 per cent.

Thrips palmi have been found successfully transmitting the virus from sunflower to sunflower (Shivasharanayya, 2000). In the present investigation, transmission was also tested with thrips to several crop plants and weed hosts. The results revealed that thrips could successfully transmit the virus disease from sunflower to cowpea, horse gram, groundnut, soybean, cucumber, water melon, field bean in addition to sunflower with the acquisition access period of more than three days and inoculation access period of 5-6 days. The symptoms varied compared to the symptom produced when mechanical sap inoculation was followed.

On groundnut (cvs., JL-24 and K-134), the symptoms produced were systemic necrotic spots on fresh emerging leaves and such symptoms to develop takes 40-45 days after inoculation access period.

On horse gram (cvs., BGM-1 and Local variety), the symptoms produced are mosaic mottling 15-20 days after inoculation access period. This was followed by reduced leaf size of infected plants, vein thickening, curling, drying of leaves and stunted growth of plants leading to tip necrosis after 25 days of inoculation.

On soybean (cv. Hardy), the symptoms produced were mosaic mottling on leaves, 10-15 days after inoculation access period, followed by chlorotic streaks on leaves and such symptoms takes 20-25 days after inoculation access period for development.

On cowpea (cvs., C-152 and Pusa Komal), symptoms were mosaic followed by chlorosis on leaves, 10-15 days after inoculation access period. This was followed by systemic necrotic spots on leaves and such symptoms to develop takes 20-25 days after inoculation and infected plants remained stunted. On field bean (Local variety), the symptoms produced were systemic, minute to small spots on leaves, followed by reduced leaf size. Such symptoms takes 25 days after inoculation access period to develop.

On cucumber (cv. Green Long), the symptoms produced as mosaic followed by chlorotic spots on leaves, 10-15 days after inoculation access period. This was followed by necrotic spots on leaves and drying of leaves and terminal shoot and such symptoms takes 15-20 days after inoculation to develop.

On water melon (cv. Arka Manik) the symptoms produced are systemic necrosis of tip of terminal buds , 15-20 days after inoculation access period. Whereas, none of the weed species tested produced symptoms even after 50 days of inoculation access period by *T. palmi* with several repetitions during the investigations.

Singh and Krishna Reddy (1995) reported *Thrips flavus* Schrank., as a new vector of tospovirus infecting water melon plants in India. Successful transmission of the virus was achieved using 10-15 nymphs with acquisition access period of 3-4 days and inoculation feeding period of 15-20 days.

Law and Moyer (1990) reported that the impatiens necrotic spot was caused by a tospovirus. The virus was reported to be transmitted by *Frakliniella occidentalis* producing necrotic spots with necrotic rings on young leaves while older leaves remained symptomless.

The per cent insect vector transmission was more in case of water melon (33.33%) followed by cowpea (26.66%), horse gram and cucumber (25%), sunflower (24%), soybean and field bean (20%) and groundnut (16.66%).

Shivasharanayya (2000) recently reported, thrips transmission of sunflower necrosis virus from sunflower to sunflower producing yellow chlorotic spots on young emerging leaves. Yellow chlorotic spots late turned necrotic, plants became stunted with the per cent transmission of 23.33 per cent. However, work done on this aspect is not available.

Future line of work

Based on the results obtained in the present investigation, further work on the following aspects could provide better understanding of the sunflower necrosis virus disease in order to develop effective management practices to contain disease.

1) Detailed survey has to be undertaken through out Karnataka to know the distribution and hot spots.
2) Purification of the virus to know precisely the nature of the particles and to study the physical and chemical properties.
3) Identification of the causal agent by serological techniques using different available antisera.
4) Detailed studies on virus-vector relationship with insect vector.
5) Relationship of tospoviruses occurring on other crops and weeds by sequence comparison.
6) Occurrence and prevalence of bio-types of *Thrips palmi* Karny, the vector of sunflower necrosis virus disease.
7) Migration, landing preference and multiplication of *T. palmi* on healthy, diseased crop and weed hosts.
8) Estimation of viruliferous *T. palmi* by sensitive detection methods at different crop growth periods on different plant species and their relation to temporal and spatial disease of the sunflower necrosis virus disease.
9) Detailed study on the host range to identify assay hosts of the causal agent.
10) Further identifying hosts of the possible weeds which may act as reservoir hosts of the disease.
11) Epidemilogical studies on spread of the disease and the vector.
12) Screening different species and sunflower germplasm for virus resistance

SUMMARY

VI SUMMARY

Results of the present investigations on sunflower necrosis virus disease with respect to survey for the incidence of the disease, symptomatology, transmission through mechanical sap inoculation, seed and insect vector and host range with emphasis on weed hosts are summarized here.

The sunflower necrosis virus disease was prevalent in sunflower fields visited during survey with the maximum percentage of incidence (21.05) on April sown crops, followed by *kharif* (10 %) and *rabi* sown crops (4.72 %).

Symptoms under field conditions start with chlorotic spots followed by necrosis of part of the leaf lamina, petiole and bud. Some of the infected plants showed systemic mosaic mottling, twisting, puckering, yellowing of leaves. Such plants are stunted with reduced internodal length compared to healthy plants. Yet some infected plants with initial necrosis found recovered from infection and produced healthy leaves and ear head but showed shortened internodal length in the middle of plant growth with normal growth after recovery.

Studies on mechanical sap transmission of the disease revealed that the disease could be transmitted through sap when potassium phosphate buffer 0.05 M (KH_2PO_4 - 0.01 M and K_2HPO_4 - 0.03 M) with 0.075 per cent thioglycerol was used as anti-inhibitor. However, the maximum transmission obtained was 45.0 per cent.

On mechanical sap inoculation to sunflower cv. Morden, symptoms appeared as twisting of freshly emerged leaves 3 to 4 days after inoculation, which produced chlorotic spots in 8-10 days. The chlorotic spots later turned necrotic which extended through the petiole and causing necrosis of terminal bud, 15-20 days after inoculation. In some of the inoculated and infected plants, only systemic mosaic mottling symptoms were observed, 20-25 days after inoculation.

Sunflower test plants of first and second pair of true leaf stages was found to be ideal for sap inoculation with increased transmission up to 45.0 per cent.

Seedlings raised from seeds collected from naturally infected plants in the field of different genotypes (Morden, KBSH-1, MSFH-17, PAC 1091, Sungene-85, CMS 234-B and RHA-6D1) did germinate with good percentage but did not show any disease symptoms even after 60 days indicating the non-seed transmission of the disease.

The vector, *Thrips palmi* was found successful in transmitting the disease when longer acquisition access period of 3 days was given under glass house conditions. The per cent transmission ranged from 12.0 to 24.0.

Out of the 70 plant species tested by mechanical sap inoculation, the virus infected sunflower, groundnut, soybean, horse gram, cucumber, water melon, cowpea, ridge guard, *Euphorbia geniculata, Galinsoga parviflora.*

On sunflower (cv. Morden and hybrids KHSH-1, PAC-1091), the virus produced mosaic symptoms followed by chlorotic spots, turning to necrosis on leaves, 10-15 days after inoculation.

On groundnut (cvs., JL-24 and K-134), the symptoms produced were mosaic followed by chlorosis on leaves, 15-20 days after mechanical sap inoculation. This was followed by systemic symptoms consisting of small to medium sized necrotic spots on subsequently emerging leaves and such symptoms to develop takes 35-40 days after inoculation.

On soybean (cv. Hardy), the symptoms produced as mosaic mottling, followed by chlorotic streaks on leaves, 20 days after mechanical sap inoculation. This was followed by systemic symptoms consisting of round necrotic spots all along the leaf margin on subsequently emerging leaves and such symptoms to develop takes 30-35 days after inoculation. Further, the infected plants remained stunted.

On cowpea (cvs., C-152 and Pusa komal), the symptoms produced as chlorosis on leaves, 8-10 days after mechanical sap inoculation. This was followed by thickening of leaf vein and there was reduction in leaf size of infected plant. Such symptoms to develop takes 15-20 days after inoculation.

On horse gram (cv., BGM-1 and Local variety), the symptoms produced as mosaic mottling, 8-10 days after mechanical sap inoculation. This was followed by reduced leaf size of infected plants vein thickening, curling and

drying of leaves and stunted growth of plants leading to tip necrosis after inoculation and such symptoms takes 10-15 days to develop.

On cucumber (cv. Green Long), the causal agent infected by producing mosaic and chlorotic spots 4 days after mechanical sap inoculation. This was followed by narrowing of leaves stunted growth and there was drying of tip of the plant resulting in apical necrosis and such symptoms take 5-6 days after inoculation to develop.

On water melon (cv. Arka Manik) the symptoms produced on mild mosaic followed by chlorosis on leaves, 5-6 days after mechanical sap inoculation. This was followed by systemic symptoms with necrosis of tip of terminal buds. It takes 7-8 days after inoculation to develop symptoms.

On ridge gourd (Local variety) the symptoms produced were mosaic followed by chlorosis, 8-10 days after mechanical sap inoculation. The infected plants remained stunted.

On *Euphorbia geniculata* symptom produced as mosaic followed by chlorotic spots on leaves, 10-15 days after mechanical sap inoculation. This was followed by curling and drying of leaves and such symptoms takes 20-25 days after inoculation to develop.

On *Galinsoga parviflora* the symptoms produced were mild mosaic followed by dark greening and vein thickening of leaves, 10-15 days after

mechanical sap inoculation. This was followed by puckering of leaves and such symptoms to develop takes 20-25 days after inoculation. There was stunted growth of plants compared to healthy plants.

Among different crop plants and weed host species tested by insect vector transmission (*T. palmi*), the virus infected groundnut, soybean, horse gram, cowpea, cucumber, water melon, field bean.

On groundnut (cvs., JL-24 and K-134), the symptoms produced as systemic necrotic spots on fresh emerging leaves and such symptoms to develop takes 40-45 days after inoculation access period.

On soybean (cv. Hardy), the symptoms produced as mosaic mottling on leaves, 10-15 days after inoculation access period, followed by chlorotic streaks on leaves and such symptoms to develop takes 20-25 days after inoculation access period.

On horse gram (cvs., BGM-1 and Local variety), the symptoms produced as mosaic mottling 15-20 days after inoculation access period. This was followed by reduced leaf size of infected plants, vein thickening, curling and there was drying of leaves and stunted growth of plants leading to tip necrosis after inoculation of 25 days.

On cowpea (cvs. C-152 and Pusa komal), produced symptoms like mosaic followed by chlorosis on leaves, 10-15 days after inoculation access

period. This was followed by systemic necrotic spots on leaves and such symptoms takes 20-25 days after inoculation to develop and infected plants remained stunted. On field bean (Local), the symptoms produced were systemic minute to small spots on leaves, followed by reduced leaf size. Such symptoms takes 25 days after inoculation access period to develop.

On cucumber (cv. Green Long), the symptoms produced were mosaic followed by chlorotic spots on leaves, 10-15 days after inoculation access period. This was followed by necrotic spots on leaves and drying of leaves and terminal shoot. Such symptoms takes 15-20 days after inoculation to develop.

On water melon (cv. Arka Manik) the symptoms produced were systemic necrosis of tip of terminal buds 15-20 days after inoculation access period. Whereas, none of the weed species tested produced symptoms even after 50 days of inoculation access period by *T. palmi* with several repetitions during the investigations.

Repeated transmission of the virus with the insect vector to 30 different weed species gave negative results, though weed species, *Galinsoga parviflora* and *Euphorbia geniculata* were successful in taking infection with mechanical sap transmission.

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